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Exploring Alternative Giant Salvinia (*Salvinia molesta* D.S. Mitchell) Management Strategies

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EXPLORING ALTERNATIVE GIANT SALVINIA (*SALVINIA MOLESTA*
D.S. MITCHELL) MANAGEMENT STRATEGIES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Plant, Environmental, and Soil Sciences

by

Bradley Todd Sartain
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M.S., Mississippi State University, 2014
May 2018

I dedicate this dissertation to my Lord and Savior Jesus Christ who led me to Louisiana State University to complete my education and blessed me with motivation during my studies. I also dedicate this to my wife Shelby, without your support and selflessness this would have never been possible. I also dedicate this to my parents, Brian and Melanie, my brother Hunter and my friends and family. Thank you for your inspirational words and support as I continued my education. I am so thankful to be surrounded by wonderful parents, family, and friends who have encouraged me to follow my passion and do what I love.

“A man’s heart plans his way, but the Lord determines his steps.” Proverbs 16:9

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Table of Contents

Acknowledgements	iii
List of Tables	vi
List of Figures	viii
Abstract	x
Chapter 1. Literature Review	1
1.1. Giant Salvinia Distribution, Biology, and Ecology	1
1.2. Methods of Control	6
1.3. Literature Cited	12
Chapter 2. Evaluation of Foliar Applied Non-Aquatic Herbicides for Control of Giant Salvinia	19
2.1. Introduction	19
2.2. Materials and Methods	22
2.3. Results and Discussion	25
2.4. Literature Cited	32
2.5. Sources of Materials	36
Chapter 3. Investigating Herbicide Resistance In Giant Salvinia Populations Exposed To Multiple Glyphosate Applications Annually	37
3.1. Introduction	37
3.2. Materials and Methods	40
3.3. Results and Discussion	43
3.4. Literature Cited	48
3.5. Sources of Materials	52
Chapter 4. Giant Salvinia Response to Foliar Herbicide Applications During Winter	53
4.1. Introduction	53
4.2. Materials and Methods	55
4.3. Results and Discussion	57
4.4. Literature Cited	64
4.5. Sources of Materials	66
Chapter 5. Effect of Winter Herbicide Applications on Bald Cypress [<i>Taxodium distichum</i> (L.) Rich.] and Giant Salvinia (<i>Salvinia molesta</i> D.S. Mitchell)	67
5.1. Introduction	67
5.2. Materials and Methods	69
5.3. Results and Discussion	72
5.4. Literature Cited	84
5.5. Sources of Materials	86

Chapter 6. Utilizing Remote Sensing Technology for Monitoring Chemically Managed Giant Salvinia Populations.....	87
6.1. Introduction.....	87
6.2. Materials and Methods.....	91
6.3. Results and Discussion	100
6.4. Literature Cited.....	107
6.5. Sources of Materials	109
Chapter 7. Summary and Conclusion	111
7.1. Literature Cited.....	115
Appendix A. Permission	116
Appendix B. Permission	117
Vita.....	121

List of Tables

Table 2.1. Registered aquatic herbicides that are efficacious on giant salvinia.	20
Table 2.2. Non-aquatic herbicides and rates (g a.i. ha ⁻¹) applied to the foliage of giant salvinia and the number of weeks until plants documented $\geq 25\%$ visual injury in herbicide screening trials 1 and 2.....	24
Table 3.1. Information collected from the Louisiana Department of Wildlife and Fisheries for waterbodies with giant salvinia populations previously managed with glyphosate in Louisiana.....	41
Table 3.2. The response of various field collected populations of giant salvinia from throughout Louisiana to foliar applications of glyphosate 8 weeks after treatment	44
Table 4.1. The response of giant salvinia (LS-mean percent control \pm SE) to the aquatic herbicides diquat, flumioxazin, glyphosate, and glyphosate + diquat applied during the winter trial 1 and trial 2.....	58
Table 4.2. Average air temperature ($^{\circ}\text{C} \pm \text{SD}$) giant salvinia was exposed to during each month of the winter trial 1 (2015-2016) and winter trial 2 (2016-2017).....	64
Table 5.1. Analysis of Variance for mean leaf length (cm) of bald cypress and percent biomass reduction of giant salvinia in response to over the top applications of aquatic herbicides	72
Table 5.2. The response of giant salvinia (LS-mean percent control) to the aquatic herbicides diquat, flumioxazin, glyphosate, and glyphosate + diquat applied at three timings during the winter 2015-2016 and 2016-2017.....	82
Table 6.1. Point intercept survey dates, number of weeks after treatment each survey was performed, the date of satellite imagery acquisition, and the corresponding sensor platform used to acquire remotely sensed data for Saline Lake, LA	94

Table 6.2. Mean near-infrared reflectance (\pm SE) and the total number of points recorded for each 10% control increment during point intercept surveys following the application of the aquatic herbicides in 2015 at Saline Lake, LA	94
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List of Figures

Figure 2.1. Dry weight biomass (mean \pm SE) response of giant salvinia 12 weeks after treatment with foliar applied non-aquatic herbicides in trial 1	27
Figure 2.2. Dry weight biomass (mean \pm SE) response of giant salvinia 12 weeks after treatment with foliar applied non-aquatic herbicides in trial 2	28
Figure 4.1. Maximum and minimum daily air temperatures ($^{\circ}$ C) A) 3 December 2015 to 20 May 2016 and B) 3 December 2016 to 20 May 2017. The symbol “T” along the x-axis represents the date of the herbicide application	59
Figure 4.2. Dry weight biomass (mean \pm SE) of giant salvinia harvested pre-treatment and from non-treated reference during the winter for trial 1 (2015-2016) and trial 2 (2016-2017).....	61
Figure 5.1. Mean leaf length of bald cypress, averaged across timing, in response to the aquatic herbicides ● diquat, ○ flumioxazin, ▼ glyphosate, Δ glyphosate + diquat, and ■ reference treatments	74
Figure 5.2. Mean leaf length (\pm SE) of bald cypress 20 weeks after bud break in response to winter applied herbicide treatments averaged across treatment and timing	75
Figure 5.3. Mean (\pm SE) leaf length of bottom, middle, and top branches of bald cypress averaged across all treatments and timings.....	79
Figure 5.4. Mean (\pm SE) bald cypress re-foliation ratings recorded every 5 weeks over the 20 week observation period following applications of aquatic herbicides	80
Figure 6.1. The 20.2 ha ⁻¹ point-intercept sampling plot on the northern shore of Chee Chee Bay in the southwestern portion of Saline Lake, LA.....	92
Figure 6.2. World View-3 true color image of the four ponds utilized in the small pond study at Cade, LA. Ponds were treated with the aquatic herbicides, glyphosate (3.3 kg a.e. ha ⁻¹) + diquat (0.5 kg a.i. ha ⁻¹) and surfactant (0.25% v/v) on 20 June 2017	96

Figure 6.3. Imagery of giant salvinia treated with the aquatic herbicides glyphosate (3.3 kg a.e. ha ⁻¹) + diquat (0.5 kg a.i. ha ⁻¹) and surfactant (0.25% v/v) at the Louisiana State University Aquaculture Research Facility in Baton Rouge, LA.....	99
Figure 6.4. Linear relationship of visual control ratings for giant salvinia treated with aquatic herbicides at Saline Lake, LA, and near-infrared reflectance values acquired from Landsat 7 and Landsat 8 satellite platforms.....	101
Figure 6.5. Linear relationship between percent control of giant salvinia treated with aquatic herbicides on 20 June 2017 at Cade, LA, and the near-infrared reflectance values acquired from WorldView-3 satellite imagery	103
Figure 6.6. Linear relationship between percent control of giant salvinia treated with aquatic herbicides on 7 September 2017 and near-infrared reflectance values collected during the mesocosm trial at Baton Rouge, LA.....	105
Figure 6.7. Linear relationship between observed percent control and predicted percent control of giant salvinia treated with the aquatic herbicides glyphosate + diquat and surfactant 6 weeks after treatment at Cade, LA	106

Abstract

Dense infestations of the free-floating aquatic fern giant salvinia (*Salvinia molesta* D.S. Mitchell) have been expanding across the southeastern United States for over two decades. Although herbicide programs have provided relief to giant salvinia infested areas, morphology and growth characteristics make management difficult. A series of mesocosm trials were conducted to explore alternative management strategies for giant salvinia. Evaluation of non-registered aquatic herbicides showed that rates of sulfometuron and metsulfuron controlled giant salvinia and provided a 98 to 100% biomass reduction. An outdoor mesocosm trial evaluated potential glyphosate resistance in giant salvinia in select water bodies throughout Louisiana. Differences between giant salvinia populations in response to glyphosate were not observed and a rate of 4.3 kg acid equivalent (a.e.) ha⁻¹ was needed for 100% control. Outdoor mesocosm trials investigated herbicide efficacy against mature giant salvinia during the winter. In trial 1, flumioxazin, glyphosate, and glyphosate + diquat provided a 45, 55, and 45% biomass reduction, respectively, in comparison to the non-treated reference. In addition, January and February applications provided a greater reduction of biomass in comparison to December applications. In trial 2, giant salvinia biomass was reduced $\geq 99\%$ for all treatments and timings in comparison to the non-treated reference. The effects of diquat, flumioxazin, glyphosate, and glyphosate + diquat over the top of bald cypress [*Taxodium distichum* (L.) Rich.] at one of three timings was evaluated. Delayed and abnormal leaf formation, reduced leaf length, irregular canopy formation, and no negative effects were observed among herbicide treated bald cypress. Biomass assessments and remotely

sensed data, collected via satellite or drone platforms, were collected to determine if these technologies could be used to accurately predict herbicide efficacy. A linear regression analyzed changes in the near-infrared reflectance signatures of chemically treated plants and observed percent control of giant salvinia plotted against predicted control yielded an $R^2 = 0.843$. These results indicate that remotely sensed data are a promising tool for monitoring large scale herbicide treatments for managing giant salvinia.

Chapter 1. Literature Review

1.1. Giant Salvinia Distribution, Biology, and Ecology

Louisiana has been at the epicenter of exotic non-indigenous aquatic plant introductions since the late 19th century. Extensive coastal wetlands, numerous freshwater systems, a sizable shipping industry, the aquarium trade, and garden/nursery industry have made the state an ideal location for establishment of invasive and non-native aquatic plant species. In most cases, these species are introduced from other parts of the world for horticulture and/or beneficial uses (Madsen 2004). Louisiana's subtropical climate and favorable growing season combined with aggressive and rapid growing plants has resulted in successful establishment. Extensive growth of nuisance aquatic plants can negatively impact ecological and economical services that Louisiana's water resources provide. Impacts can include: obstructing navigation/irrigation canals, lowering property values, reducing recreational use of rivers and lakes, disrupting nutrient cycling, and altering the interactions between fish and other aquatic species (Pimentel et al. 1999, Madsen 2004).

Since the introduction of water hyacinth [*Eichhornia crassipes* (Mart) Solms] to New Orleans during the 1884 Cotton States Exposition (Wolverton and McDonald 1979), Louisiana has been hindered by nuisance aquatic plants. In addition to water hyacinth, other nuisance aquatic plant species, including hydrilla (*Hydrilla verticillata* R.f. Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.), soon found their way into Louisiana waters (Horst and Mapes 2000). During the past two decades, a new aggressive invader, giant salvinia (*Salvinia molesta* D.S. Mitchell) has established in the

state of Louisiana and Texas. It was first discovered in Louisiana in 1998 on the Toledo Bend Reservoir, which lies on the Louisiana-Texas border (Horst and Mapes 2000). It has since spread across eastern Texas and to the majority of water bodies in Louisiana. Due to its rapid growth, giant salvinia has been deemed one of the world's worst aquatic weeds (Holm et al. 1977) and has caught the attention of water resource managers across the southern United States.

Giant salvinia is a free-floating aquatic fern that originates from Brazil (Jacono 1999, Jacono and Pittman 2001, McFarland et al. 2004). Over the past 80 years, giant salvinia has migrated outside of its native range in South America (Oliver 1993, Jacono and Pittman 2001) to Africa (Mitchell and Tur 1975, Cilliers 1991), India (Cook 1976), Sri Lanka (Room 1990), Southeast Asia (Baki et al. 1990), Australia (Forno and Harley 1979), and the United States (Johnson 1995). The ornamental plant trade is most likely the vector for the intercontinental spread of giant salvinia. Botanical gardens and commercial horticulture have been linked to its introduction in other parts of the world (Room et al. 1981), and its discovery and eradication has been documented at aquatic plant nurseries in Florida, allegedly in a contaminated aquatic plant shipment from Sri Lanka (Nelson 1984). The first reported documentation of established giant salvinia in the United States occurred in 1995 in South Carolina (Johnson 1995). Since 1995, it has been found in an additional eleven states including Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, Texas, and Virginia (Thayer et al. 2018).

Giant salvinia belongs to the family Salviniaceae, which is characterized as free-floating aquatic ferns that possess velvety hairs on the leaf surface (McFarland et al.

2004). Giant salvinia along with three other salvinia species (*S. auriculata*, *S. herzogii*, and *S. bilboa*) exhibit rows of “cylindrical hairs” or trichomes that form an “egg beater” or “cage-like” structure on the leaf surface (Forno 1983, McFarland et al. 2004). Giant salvinia forms floating colonies of autonomous modules or ramets (McFarland et al. 2004), and each plant is held together by a horizontal stem (rhizome) below the water surface. There are three distinct fronds (leaves) along with 4 to 5 inconspicuous buds on each ramet. Two of the fronds (either emergent or floating) are ovate to oblong, while a modified third frond is submerged underwater. The surface of the emergent or floating fronds are covered by rows of white trichomes (McFarland et al. 2004) that are capable of repelling water and aid with floatation (Harper 1986). Giant salvinia does not have a true “root,” but submersed fronds, which are brown, highly dissected, and absorb nutrients from the water column (McFarland et al. 2004). Plants can also develop large quantities of long chain sporocarps among filaments of the submersed fronds (Loyal and Grewal 1966). Sporocarps are structures that contain the fern’s non-viable spores and these structures are often developed (if at all) later in the growing season or during nutrient poor conditions (Nelson 2014).

Giant salvinia exhibits three main growth forms: primary, secondary, and tertiary. The primary stage occurs during the initial invading stage and consists of secluded or widely spaced plants with small, fragile, oval leaves (10 to 15 mm wide) that lie flat across the water surface (Harley and Mitchell 1981). The primary growth stage can also be observed in plants recovering from damage and/or uncrowded conditions (Harley and Mitchell 1981). The secondary stage is typically observed in plants that have been growing in open water for some time. In comparison, secondary growth stage stem

internodes are longer and more robust, fronds (20 to 50 mm) begin to cup slightly, and the lower frond surface makes contact with the water (McFarland et al. 2004). The tertiary stage, also called the mat stage, occurs under crowded conditions. Fronds are large (up to 60 mm), and as plants become more crowded, the fronds are directed upward and have been documented to create mats that are two or more plant layers and may be up to 1 meter thick (McFarland et al. 2004).

Giant salvinia reproduction is believed to be completely asexual. The plant is characterized as pentaploidal or having a chromosome number five times that of a haploid, thus making it genetically incapable of producing viable spores (Loyal and Grewal 1966). Fragmentation can occur very easily, and is believed to be the main factor leading to the spread of this species. Parent plants can break at the rhizome and lead to the formation of one or more daughter plants (Room 1983). Fragmentation and transport of giant salvinia can be caused by human/animal influence and/or environmental influences, such as wind and wave currents (Harley and Mitchell 1981, Room 1983, 1990).

Giant salvinia exhibits very rapid growth, with plant biomass doubling under greenhouse conditions in only 2.2 days (Cary and Weerts 1983). Conversely, field observations have shown the amount of time for a two-fold increase in plant biomass to range from 1 to 8 days (Finlayson 1984, Room 1986). Plant coverage increased 100% in 2.2 to 2.7 days during midsummer and 40 to 60 days during winter in Lake Moondara, Australia (Farrel 1979, Harley and Mitchell 1981, Finlayson 1984). It was also reported that plant coverage increased 100% in Lake Kariba, Africa in 8.1 days during midsummer and 14.3 days during winter (Mitchell and Tur 1975). In addition to seasonal

growth differences, they reported that when giant salvinia growth was averaged across all seasons, secondary growth increased biomass faster (8.6 days) than the tertiary form (11.6 days).

Water quality parameters such as temperature, pH, and available nutrients impact giant salvinia growth rate and survival. Room (1988) developed a predictive model to show the relationship between giant salvinia growth as it relates to temperature. The model demonstrates plant growth is at a maximum level near 30° C and has upper and lower limits at 40° and 5° C, respectively. Several studies have examined the influence of pH in response to giant salvinia growth. When evaluated over a pH range of 5 to 8, Cary and Weerts (1984) demonstrated that biomass production was greatest at pH 6. Owens et al. (2005) also documented increased plant growth when water pH was maintained < 7.5 when compared to pH > 8.5 at research ponds in Lewisville, Texas. Madsen and Wersal (2008) analyzed the effect of water pH and nitrogen additions on giant salvinia biomass and documented when no nitrates were present, pH was a significant factor limiting giant salvinia growth up to 14 days after establishment (DAE) of the experiment. In contrast, no differences were detected among pH and biomass production 84 DAE. Thus, pH may not be a factor regulating the long term growth of giant salvinia, but more so during the early colonizing stages or when nutrients are limited. Madsen and Wersal (2008) also documented giant salvinia growth was significantly affected by the addition of nutrients to the water column, with both nitrogen treatments (9.0 mg L⁻¹ and 13.5 mg L⁻¹) significantly increasing biomass when compared to plants that did not receive nitrogen.

1.2. Methods of Control

Due to giant salvinia's rapid growth and ability to form large extensive mats, water resources have been impacted immediately after the initial infestation. Dense plant growth impedes navigation, irrigation, and recreational use of infested water bodies (Pimentel et al. 1999) leading to not only environmental impacts, but economic impacts and public health concerns (McFarland et al. 2004). Physical, biological, chemical, and integrated control methods are the management options available to control giant salvinia.

1.2.1. Physical Control

Physically removing the plant by hand, utilizing a mechanical harvester that inflicts damage to the plant by cutting/shredding/chopping, deploying a barrier to prevent the plant from spreading or encroaching, and modifying the environment to reduce or eliminate suitable plant habitat are all physical control options (Chilton et al. 2002). Manual removal by hand or a harvester has worked well in small single layer infestations (Thomas and Room 1986, Miller and Wilson 1989), but once the plant is well established, this option is less practical. Manually removing the plant by hand is very labor intensive and impractical for large infestations. Mechanical harvesters have high operating costs, roughly \$3,400 to \$3,700 per hectare (B. Hartis, personal communication, 2016), and must be capable of removing and disposing of the harvested biomass in a timely manner that exceeds the rapid growth of giant salvinia (Storrs and Julien 1996). Cutting, chopping, or shredding is probably the least effective method of plant removal since giant salvinia reproduces by fragmentation. Physical barriers, such as booms and nets, are suitable methods to prevent plant movement to surrounding areas,

but are often labor intensive and require continuous maintenance and inspection (Oliver 1993). Habitat alteration, such as lake drawdown, has been an effective tool for managing giant salvinia and other aquatic plant species (Bellaud 2014). The intent of a drawdown is to control the target plant by exposing it to dry land and drying it out and/or exposing it to freezing temperatures (Cooke et al. 1986), as well as, decreasing the number of acres to chemically manage. Success using this technique is dependent on the growth stage of the plant. Extensive giant salvinia mats several layers thick may be able to insulate and protect viable ramets during drawdowns, thus allowing the colony to regenerate when favorable conditions return (McFarland et al. 2004).

Lake drawdowns have been effective at decreasing giant salvinia acreage in Lake Bistineau, Louisiana, and have been utilized annually since 2008 (Louisiana Department of Wildlife and Fisheries 2013). Map surveys conducted by Louisiana Department of Wildlife and Fisheries (LDWF) have shown that lake drawdown decreased plant coverage from 1,800 hectares in July of 2008 to 340 hectares in February 2009 (Louisiana Department of Wildlife and Fisheries 2013). Consequently, drawdowns can also cause conflicts from stakeholders since shallow water may impact lake access and/or recreational activities (i.e. fishing, hunting, and boating) (Chilton et al. 2002).

1.2.2. Biological Control

The salvinia weevil (*Cyrtobagus salviniae* Calder and Sands) has shown favorable results as a biological control agent, and has substantially reduced plant populations in Australia (Room 1986, Creagh 1991/1992, Sullivan et al. 2011), Sri Lanka (Room et al. 1989), India (Joy et al. 1986), South Africa (Cilliers 1991), and Botswana (Julien and Griffiths 1998). The weevils are very host specific and will utilize giant salvinia for

feeding and reproduction over other available plants (Forno et al. 1983). Both the adult and larval growth stages cause plant damage, but it is believed that the larvae are the most destructive (Sands et al. 1983). Although the weevil has shown success in southern Louisiana, it has limitations in the central and northern portions of the state. Releases of salvinia weevils in northern Texas and Louisiana from 2009 to 2011 were unsuccessful due to historically cold winters that decimated previously established populations (Grodowitz 2011, Sanders 2011). In contrast, overwintered populations of salvinia weevils were observed in northern Louisiana during 2015 (Cozad 2017). Research has documented that salvinia weevil ecotypes vary in their ability to tolerate cold conditions (Mukherjee et al. 2014, Russell et al. 2017) and a single salvinia weevil genotype is capable of adapting to localized conditions (Cozad 2017). Biological control with salvinia weevils; however, can take a considerable amount of time. Julien and Griffiths (1998) documented that weevils required 1 to 5 years to control giant salvinia in Botswana, Africa.

1.2.3. Chemical Control

Herbicides are one of the most commonly used management tools in aquatic plant management (Netherland 2014). Currently, fourteen active ingredients are registered for use in and around aquatics systems by the United States Environmental Protection Agency (USEPA) (Netherland and Jones 2012, University of Florida 2014). Ten of the fourteen registered active ingredients have activity on giant salvinia including: bispyribac-sodium, carfentrazone-ethyl, copper, diquat dibromide, endothall, flumioxazin, fluridone, glyphosate, penoxsulam, and topramezone (Nelson et al. 2001,

Glomski et al. 2003, Glomski and Getsinger 2006, Glomski and Mudge 2013, Mudge and Harms 2012, Mudge et al. 2012, 2013, Mudge 2016).

Carfentrazone-ethyl, diquat, endothall, and flumioxazin are contact herbicides that are effective when applied to the foliage of single layer mats of giant salvinia (Nelson et al. 2001, Glomski and Getsinger 2006, Richardson et al. 2008). Contact herbicides are rapid acting and injury symptoms and plant death is typically documented three to four days after treatment (Nelson 2014), especially when applied at higher rates.

Unfortunately, contact herbicides do not translocate throughout the plant and only tissue contacted with the herbicide will be affected, which is problematic in dense infestations where underlying plants are not exposed to the herbicide solution.

Systemic herbicides such as glyphosate, fluridone, bispyribac-sodium, penoxsulam, and topramezone are translocated throughout the plant, but injury symptoms are more gradual (Nelson 2014). Glyphosate can only be effective if applied to plant foliage because it is rapidly inactivated in natural waters (Carlisle and Trevors 1988). Penoxsulam and bispyribac-sodium can be applied as a foliar spray or subsurface applications (i.e. applied to the water column) (Mudge et al. 2012, Glomski and Mudge 2013, Mudge 2016), whereas topramezone is more effective as a foliar application (Mudge 2016) and fluridone is primarily effective as a subsurface application (Mudge et al. 2012).

Systemic herbicides applied subsurface require longer exposure times (days to weeks) than contact herbicides (hours to days), thus control can be difficult in fluvial systems with high water exchange. Rapid or minimal water exchange in rivers, canals, or reservoirs can result in movement of the herbicide out of the application area, thus

preventing necessary plant exposure to a lethal dose of the herbicide. Mudge et al. (2012) documented that penoxsulam and fluridone were effective against giant salvinia if lethal herbicide concentrations are maintained for at least 12 weeks. In addition, Glomski and Mudge (2013) documented significant reductions of giant salvinia biomass with subsurface applications of bispyribac-sodium [40 to 80 μg active ingredient (a.i.) L^{-1} (ppb)]; however, plant recovery occurred and they concluded that multiple applications per growing season would be necessary to achieve control.

In 2009, LDWF performed a large scale subsurface application of penoxsulam (20 μg a.i. L^{-1}) to control 526 hectares of giant salvinia in several areas of Lake Bistineau, Louisiana (Louisiana Department of Wildlife and Fisheries 2013). Water samples collected post application to assess penoxsulam concentration within the treatment sites indicated that the herbicide concentration had dropped significantly and fell below the lethal concentration within the majority of the treated areas two weeks after application, and consequently resulted in minimal giant salvinia control (Louisiana Department of Wildlife and Fisheries 2013). The high cost of subsurface treatments and the probability of not maintaining a lethal dose for an extended period of time makes foliar herbicide applications the most utilized application method.

Small scale research and large-scale field operations have demonstrated that aquatic herbicides are capable of managing giant salvinia (McFarland et al. 2004). To date, glyphosate and diquat applications (alone and combination treatments) are the most utilized and effective for giant salvinia control (McFarland et al. 2004, Mudge et al. 2016), but other recently registered herbicides such as carfentrazone-ethyl, flumioxazin, bispyribac-sodium, penoxsulam, and topramezone have demonstrated efficacy when

applied alone or in combination with other chemistries (Glomski and Getsinger 2006, Mudge and Harms 2012, Mudge et al. 2012, Glomski and Mudge 2013, Mudge 2016, Mudge et al. 2016). With a limited number of herbicides currently available for giant salvinia management, it is imperative that other herbicides with different modes of action are evaluated as control options and tools for managing giant salvinia. In addition, use of herbicides with different modes of action should be routinely rotated or used in combination as a best management practice to help reduce the potential for herbicide resistance (Nelson 2014).

To date, herbicide resistance in giant salvinia has not been documented; however, multiple infestations throughout Louisiana have been exposed to glyphosate annually for over a decade (C. Mudge, personal communication, 2018). The repeated use of glyphosate as the primary means of chemical weed control has led to 39 glyphosate resistant plant species worldwide (Heap 2017). Giant salvinia populations that have received multiple glyphosate applications should be evaluated for response to glyphosate.

Although herbicide programs have provided relief to numerous areas infested with giant salvinia, its morphology and growth characteristics will make chemical control measures difficult. The plants are small, floating, and have numerous trichomes on the leaf surface that repel water, thus making it difficult to achieve herbicide to plant contact and herbicide uptake (Holm et al. 1977, Thayer and Haller 1985, Oliver 1993). Fronds beneath the water surface that do not come in direct contact with the herbicide solution increase the difficulty of obtaining control. In addition, many giant salvinia infestations occur in lakes and swamps with dense bald cypress [*Taxodium distichum* (L.) Rich.] stands that make it difficult for herbicide applicators to not only access healthy plant

populations, but achieve adequate herbicide coverage. Research focusing on herbicide application timing could provide beneficial data critical to the development of efficient management programs. Herbicide applications in late fall through late winter, when plant biomass may have been reduced by lower temperatures and freezes, may result in greater efficacy compared to spring and summer applications. Lastly, the mobility of giant salvinia makes it difficult to monitor herbicide efficacy in operational management scenarios. Factors such as wind movement, wave action, fluctuating water levels, and human influence all provide a means of plant transport in and out of treated areas. Research efforts are needed to improve or develop new monitoring techniques to assess large-scale herbicide applications in an operational setting.

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Chapter 2. Evaluation of Foliar Applied Non-Aquatic Herbicides for Control of Giant Salvinia

2.1. Introduction

Giant salvinia (*Salvinia molesta* D.S. Mitchell) is a free-floating aquatic fern that originates from Brazil (Jacono 1999, Jacono and Pitman 2001, McFarland et al. 2004). Over the past 80 years, giant salvinia has migrated outside of its native range in South America (Oliver 1993, Jacono and Pittman 2001) to Africa (Mitchell and Tur 1975, Cilliers 1991), India (Cook 1976), Sri Lanka (Room 1990), Southeast Asia (Baki et al. 1990), Australia (Forno and Harley 1979), and the United States (Johnson 1995). The first reported documentation of giant salvinia established in the United States occurred in 1995 in South Carolina (Johnson 1995). Since 1995, it has been found in an additional eleven states including Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, Texas, and Virginia (Thayer et al. 2018).

Giant salvinia exhibits very rapid growth with plant biomass doubling under greenhouse conditions in only 2.2 days (Cary and Weerts 1983). Conversely, field observations have shown the amount of time for a two-fold increase in plant biomass to range from 1 to 8 days (Finlayson 1984, Room 1986). In addition, it forms dense mats made up of multiple plant layers, which have been documented up to 1 meter thick (McFarland et al. 2004). Dense plant growth impedes navigation, irrigation, and recreational use of infested water bodies (Pimentel et al. 1999) leading to not only environmental impacts, but economic impacts and public health concerns (McFarland et al. 2004). These negative impacts have led to situations where giant salvinia needs to be intensively managed to limit its growth and spread to surrounding water bodies.

Small scale research and large-scale field operations have shown that aquatic herbicides are capable of managing giant salvinia infestations in the United States (McFarland et al. 2004). Currently, fourteen active ingredients are registered by the United States Environmental Protection Agency (USEPA) for use in or around aquatic sites; however, ten have activity on giant salvinia (Table 2.1) (Nelson et al. 2001, Glomski et al. 2003, Glomski and Getsinger 2006, Glomski and Mudge 2013, Mudge and Harms 2012, Mudge et al. 2012, 2013, Mudge 2016).

Table 2.1. Registered aquatic herbicides that are efficacious on giant salvinia.

Herbicide	Mode of Action	Application Method
Bispyribac-sodium	Acetolactate Synthase Inhibitor (ALS)	Foliar/Subsurface
Carfentrazone-ethyl	Protoporphyrinogen Oxidase Inhibitor (PPO)	Foliar/Subsurface
Copper	Not Classified	Foliar/Subsurface
Diquat	Photosystem I	Foliar/Subsurface
Endothall	Not Classified	Foliar/Subsurface
Flumioxazin	Protoporphyrinogen Oxidase Inhibitor (PPO)	Foliar/Subsurface
Fluridone	Carotenoid Biosynthesis Inhibitor (Phytoene Desaturase)	Subsurface
Glyphosate	Enolpyruvyl Shikimate-3-Phosphate (EPSP) Synthase Inhibitor	Foliar
Penoxsulam	Acetolactate Synthase Inhibitor (ALS)	Foliar/Subsurface
Topramezone	Carotenoid Biosynthesis Inhibitor (HPPD)	Foliar

To date, glyphosate and diquat applications are the most effective for giant salvinia control (Mudge et al. 2016), but other herbicides including carfentrazone-ethyl, flumioxazin, bispyribac-sodium, penoxsulam, and topramezone have demonstrated varying levels of control when applied alone or in combination with other chemistries (Glomski and Getsinger 2006, Mudge and Harms 2012, Mudge et al. 2012, Glomski and Mudge 2013, Mudge 2016, Mudge et al. 2016).

A combination of glyphosate [3.4 kg acid equivalent (a.e.) ha⁻¹], diquat [0.5 kg active ingredient (a.i.) ha⁻¹], and two surfactants has been used almost exclusively for giant salvinia control by the Louisiana Department of Wildlife and Fisheries (LDWF) (Mudge et al. 2016) as well as other Federal and state agencies in Louisiana and Texas (C. Mudge, personal communication, 2018). Despite this combination being effective against giant salvinia, the continuous use of one herbicide or one spray mixture will be detrimental if giant salvinia were to develop resistance to either herbicide in the future (Mudge et al. 2016). Although herbicide resistance in aquatic weed management has been limited to fluridone resistant hydrilla (*Hydrilla verticillata* L.f. Royle) (Michel et al. 2004) and diquat resistant landoltia duckweed [*Landoltia punctata* (G. Meyer) D.H. Les and D.J. Crawford] (Koschnick et al. 2006), over 250 herbicide resistant plant species have been documented globally at a rate of 11 new cases per year (Heap 2014, Heap 2017). Best management practices that promote the rotation of herbicides are encouraged to decrease the chances of establishing resistant plant populations. As a result of the overuse of two active ingredients and a limited number of efficacious aquatic herbicides, it is important to evaluate other potential chemistries (i.e. herbicides with different modes of action) and/or non-aquatic herbicides.

Herbicide screenings for giant salvinia have been limited because it is considered a regional weed compared to more widespread invasive aquatic species, such as hydrilla and Eurasian watermilfoil (*Myriophyllum spicatum* L.). Regional weed problems represent a small market for the herbicide industry and essentially it is not economically beneficial for agro-chemical companies to develop new chemistries for a regional weed species. Bispyribac-sodium, carfentrazone-ethyl, flumioxazin, imazamox, penoxsulam, and topramezone were registered for aquatic use after fluridone-resistant hydrilla was discovered in the late 1990's (Haller 2011, Haller and Gettys 2017). The objectives of this research were to 1) evaluate the efficacy of 12 non-aquatic herbicides against giant salvinia and 2) further evaluate products that provided $\geq 30\%$ control at additional rates.

2.2. Materials and Methods

Two outdoor mesocosm trials were conducted and repeated at the Louisiana State University (LSU) Aquaculture Research Facility in Baton Rouge, LA, in 2016 (July and September) and 2017 (May and August). In trial 1, the efficacy of twelve non-aquatic herbicides was evaluated against giant salvinia. In trial 2, herbicides that provided at least a 30% reduction of giant salvinia biomass in trial 1(excluding clomazone) were re-evaluated at additional rates. Giant salvinia used in this research was collected from cultures maintained at LSU Aquaculture.

In both trials, plants were cultured in 76 L plastic containers (49.5 cm diameter by 58.4 cm height) filled with 60 L of pond water (pH 8.5). Prior to planting, pond water was amended with sphagnum peat moss (14 g) to lower the pH to < 7.0 . Equal amounts of fresh plant material, enough to cover approximately 85% of the water surface, were

placed in each 76 L container. In addition, 2.1 g of Miracle-Gro®¹ Water Soluble Lawn Food (24-8-16) was applied to each container at planting and every 2 weeks throughout both trials to encourage plant growth. Plants were allowed to acclimate to container conditions for 2 weeks prior to herbicide application. At herbicide application, plants had reached ca. 100% coverage with mean dry weights of 33.76 ± 1.59 and 28.72 ± 2.03 g for trial 1 and 2, respectively. Culture and planting techniques were adapted from previous giant salvinia research (Nelson et al. 2007, Mudge et al. 2012, Mudge et al. 2016).

Herbicides evaluated during the trial 1 and trial 2 included acetolactate synthesis inhibitors (ALS): bensulfuron², halosulfuron³, metsulfuron⁴, rimsulfuron⁵, sulfometuron⁶, trifloxysulfuron⁷; protoporphyrinogen oxidase inhibitors (PPO): saflufenacil⁸ and flumiclorac⁹; carotenoid biosynthesis inhibitor: clomazone¹⁰; glutamine synthesis inhibitor: glufosinate¹¹; synthetic auxin: florypyrauxifen-benzyl¹²; and acetyl CoA carboxylase (ACCase) inhibitor: sethoxydim¹³ (Table 2.2).

All herbicides were applied at maximum application rates based on the USEPA Section 3 label in their respective use sites (i.e. row crops, horticulture, turf, right-of-way, etc.) for trial 1 and additional rates in trial 2. Each treatment included a modified vegetable oil and non-ionic organosilicon surfactant blend¹⁴ at 0.25% v/v. A non-treated reference was also included. A completely randomized design was utilized with 4 replicates per treatment. Herbicide treatments were applied to the foliage of giant salvinia using a CO₂-powered sprayer at an equivalent of 935 L ha⁻¹ diluent delivered through a single TeeJet®¹⁵ 80-0067 nozzle at 20 psi. To evaluate herbicide efficacy all viable plant biomass were harvested 12 weeks after treatment (WAT) and dried (65° C). Dry weight biomass from each trial were subjected to an analysis of variance (ANOVA)

using Proc Glimmix procedure in SAS® version 9.3 (2017) statistical software with trial replicates as a random effect. Means were separated using Fishers Protected LSD test ($p \leq 0.05$).

Table 2.2. Non-aquatic herbicides and rates (g a.i. ha⁻¹) applied to the foliage of giant salvinia and the number of weeks until plants documented $\geq 25\%$ visual injury in herbicide screening trials 1 and 2.

Herbicide Treatments	Rate	$\geq 25\%$ visual injury
<i>Trial 1</i>	g a.i. ha ⁻¹	WAT
Bensulfuron	70	2
Clomazone	1393	2
Flumiclorac	60	1
Glufosinate	882	1
Halosulfuron	290	2
Metsulfuron	42	2
Florpyrauxifen-benzyl	117	N/A
Rimsulfuron	35	N/A
Saflufenacil	150	1
Sethoxydim	526	N/A
Sulfometuron	315	2
Trifloxysulfuron	21	3
<i>Trial 2</i>		
Bensulfuron	70	3
	140	3
Halosulfuron	290	3
	580	2
Metsulfuron	21	3
	42	3
	84	2
Saflufenacil	300	1
Sulfometuron	158	3
	315	2
	630	2
Trifloxysulfuron	42	3

Abbreviations: a.i., active ingredient; N/A, not applicable indicated that plant injury never exceeded 25%.

2.3. Results and Discussion

The non-aquatic herbicides evaluated as foliar applications against giant salvinia provided a variety of injury symptoms. In trial 1, injury to giant salvinia from saflufenacil, flumiclorac, and glufosinate resulted in chlorosis and necrosis to giant salvinia less than 1 WAT. Despite saflufenacil and flumiclorac having the same mode of action (PPO inhibitors), saflufenacil resulted in faster injury symptoms than flumiclorac. Although rapid plant injury was documented with the aforementioned products, plant recovery was evident 2 WAT and all three herbicide treatments failed to provide $\geq 35\%$ control 12 WAT (Figure 2.1).

Clomazone treated plants exhibited bleaching and chlorosis less than 2 WAT with peak injury observed 5 WAT. Halosulfuron and bensulfuron treatments exhibited chlorosis of existing fronds and growth reduction of newly formed fronds 2 WAT; plants were necrotic by 4 WAT. Unfortunately, plant recovery was documented ≤ 6 WAT and was very noticeable by the conclusion of the 12 week study in clomazone, halosulfuron, and bensulfuron treatments, which resulted in 69, 76, and 77% control, respectively. Florpyrauxifen-benzyl caused short-term and minimal visible injury ($\leq 10\%$) with newly formed fronds appearing slightly stunted 3 WAT; however, no injury symptoms were visible at 5 WAT. Sethoxydim and rimsulfuron treatments did not produce any visible injury symptoms and dry weight biomass were not significantly different from reference treatments at the conclusion of trial 1. Metsulfuron and sulfometuron were the most effective treatments and provided 100% control of giant salvinia at the end of the 12 week study (Figure 2.1). Both treatments caused plants to become necrotic, lose buoyancy, and desiccate as early as 2 WAT. At 6 WAT, $\geq 85\%$ of the plant material fell

below the water surface and deteriorated, and by 7 WAT only a few small chlorotic and necrotic fronds remained at the water surface. By 8 WAT, 100% plant mortality was documented for both treatments.

Due to the level of control provided by bensulfuron, halosulfuron, metsulfuron, saflufenacil, sulfometuron, and trifloxysulfuron in trial 1, these products were further evaluated in trial 2 at additional rates. All herbicide treatments in trial 2 reduced giant salvinia biomass 53 to 99% in comparison to the non-treated reference 12 WAT (Figure 2.2). The rate of saflufenacil (300 g a.i. ha⁻¹) in trial 2 provided better control (89%) compared to trial 1 (31%, 150 g a.i. ha⁻¹); however, plant recovery was observed 3 WAT. The 2x rate of trifloxysulfuron in trial 2 provided 53% control, which was modestly higher than the 44% control observed in trial 1. There were no differences in plant response between plants treated with the 1x or 2x rate of bensulfuron or halosulfuron in trial 2 with each providing 92 to 96% control, respectively. All three rates of metsulfuron and sulfometuron provided 98 to 99% control.

Saflufenacil (trial 1 and 2) and flumiclorac (trial 1) performed similar to the contact herbicides flumioxazin and carfentrazone-ethyl, which are also PPO inhibiting herbicides currently registered for aquatic use by the USEPA (Netherland 2014). Flumioxazin and carfentrazone-ethyl are efficacious when applied to the foliage of giant salvinia (Glomski and Getsinger 2006, Richardson et al. 2008); however, regrowth frequently occurs, especially when multiple plant layers are present (Mudge et al. 2016). These previous findings (Mudge et al. 2016) are similar to observations documented in the current studies with saflufenacil and flumiclorac treated plants documenting recovery ≤ 3 WAT.

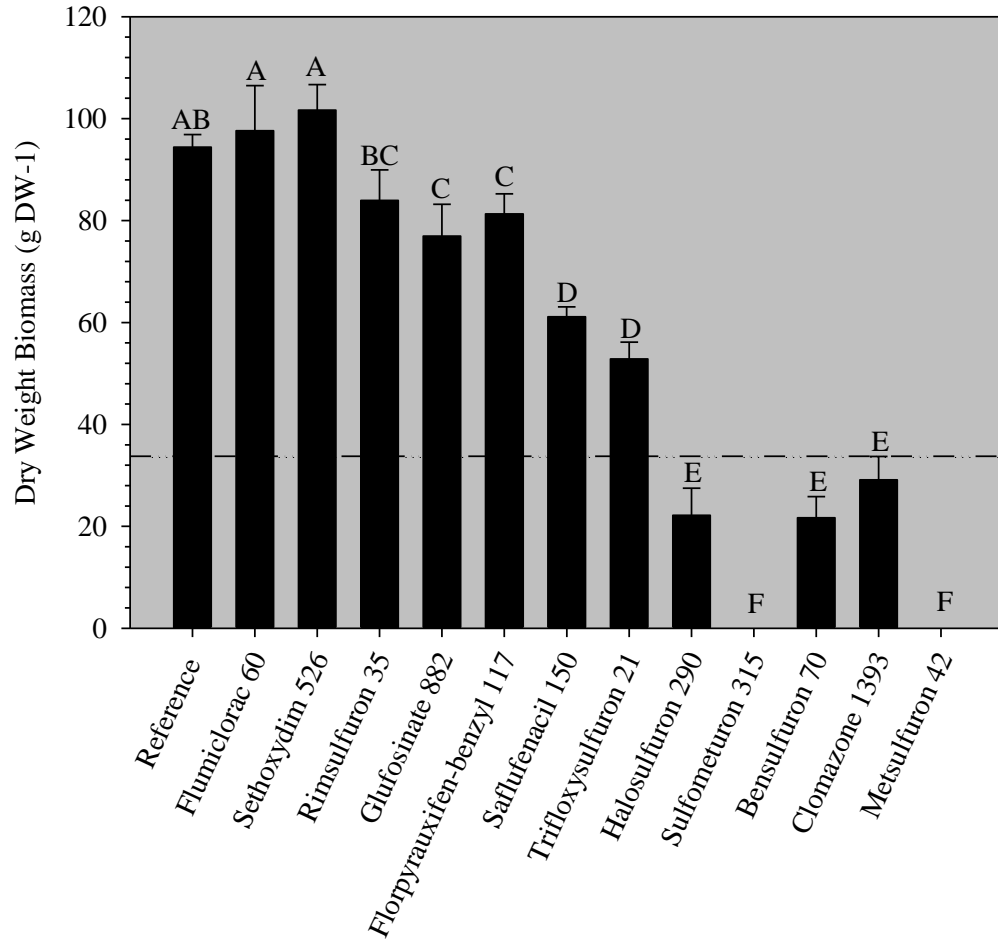


Figure 2.1. Dry weight biomass (mean \pm SE) response of giant salvinia 12 weeks after treatment with foliar applied non-aquatic herbicides in trial 1. Treatments sharing the same letter are not significantly different according to Fisher's Protected LSD test $p \leq 0.05$; $n = 8$. Horizontal dashed line represents pre-treatment biomass. Numbers following the treatment represent g a.i. ha⁻¹.

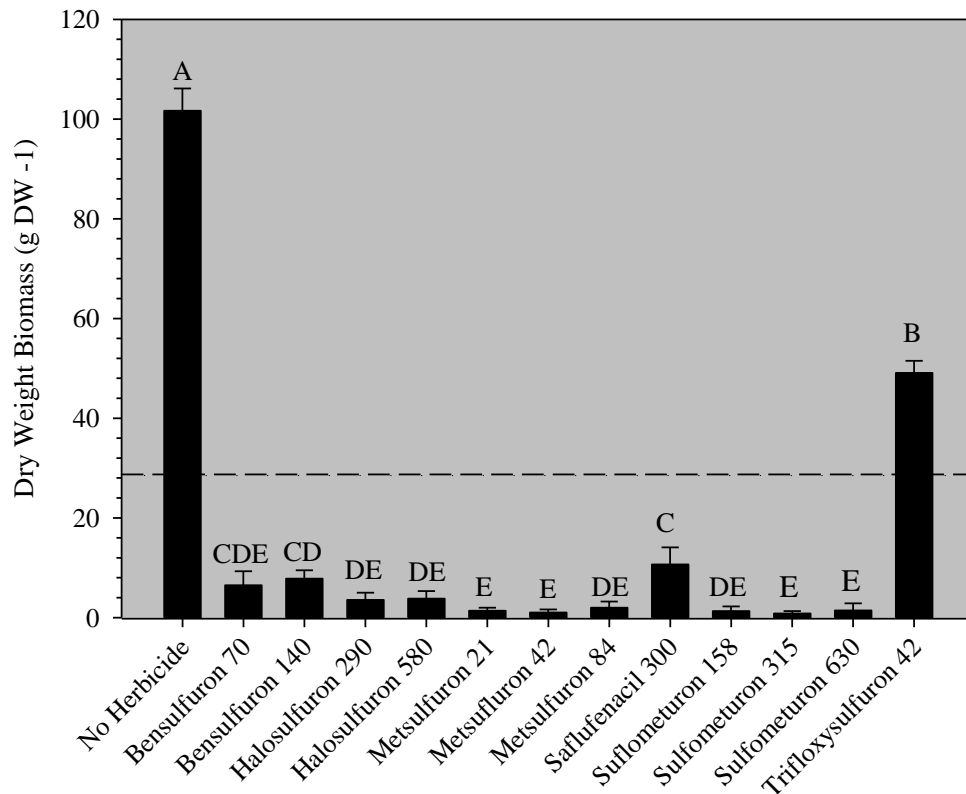


Figure 2.2. Dry weight biomass (mean \pm SE) response of giant salvinia 12 weeks after treatment with foliar applied non-aquatic herbicides in trial 2. Treatments sharing the same letter are not significantly different according to Fisher's protected LSD test $p \leq 0.05$; $n = 8$. Horizontal dashed line represents pre-treatment biomass. Numbers following each treatment represent herbicide rates in g a.i. ha^{-1} .

Clomazone, halosulfuron, and bensulfuron are currently registered by the USEPA and labeled for use in several row crops for control of broadleaf weeds, sedges, and grass species (Shaner 2014). Giant salvinia exposed to bensulfuron and halosulfuron documented typical ALS herbicide symptomology. Fronds that developed after herbicide application appeared crinkled, stunted, and chlorotic, whereas older fronds gradually became necrotic, lost buoyancy, and deteriorated. These injury symptoms are similar to the registered aquatic herbicide penoxsulam, which is also an ALS inhibitor that is efficacious against giant salvinia as a foliar or subsurface application (Mudge et al.

2012). Bensulfuron has previously been documented as efficacious against fern species. Toxicity tests of bensulfuron against the aquatic ferns *Salvinia natans* (L.) All., *Azolla japonica* Franch. & Sav., and *Marsilea quadrifolia* (L.) indicated an ED₅₀ (estimated dose to kill 50% of the test population) of less than 1/20th the recommended application rate (51 to 75 g a.i. ha⁻¹) for bensulfuron in Japanese rice paddy fields (Aida et al. 2004). Although clomazone efficacy was observed in trial 1, it was excluded from trial 2 because of potential volatility issues and off-site injury of sensitive plants (Mervosh et al. 1995) that may decrease the probability of achieving an aquatic label for giant salvinia control.

It is evident from the results of these screenings that sulfometuron and metsulfuron are highly efficacious against giant salvinia. Both treatments provided noticeable visual injury symptoms ≤ 2 WAT and 98 to 100% plant control as early as 8 WAT across all the rates evaluated. Although sulfometuron and metsulfuron did not provide 100% giant salvinia control in trial 2, no new fronds were observed and harvested material consisted of small rhizome fragments that had limited viability.

Sulfometuron is registered for use in conifer and hardwood sites for the control of annual and perennial broadleaf and grass species, and late fall/early winter applications in unimproved turf sites (Shaner 2014). Sulfometuron activity on non-aquatic fern species has been previously documented. Horsley (1988) reported 100% control of hay-scented fern (*Dennsataedtia punctilobula* Michx. Moore) and New York fern (*Thelypteris noveboracensis* L. Nieuwl.) two years after applications of sulfometuron at 102, 204, and 408 g a.i. ha⁻¹. In addition, sulfometuron and tank mixes of sulfometuron + glyphosate

have been successfully implemented to reduce hay-scented fern densities in forested areas of the northeastern United States (Fei et al. 2010).

Metsulfuron is registered for control of broadleaf weeds in grain crops, pasture grass species (Shaner 2014), turf grass, and brush control (Bayer 2017a). In addition, metsulfuron can be applied under the authority of a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Special Local Need (SLN) 24(c) label to control old world climbing fern [*Ligodium microphyllum* (Cav.) R. Br; OWCF] and for use in lake restoration projects in dewatered zones of lakes in Florida (Bayer 2017b, Bayer 2017c). The efficacy of metsulfuron on OWCF subsequently led to the testing of metsulfuron on giant salvinia in the current herbicide screening trials. Langeland and Link (2006) documented 100% control of OWCF with foliar applications of metsulfuron at 40 and 80 g a.i. ha⁻¹, which is comparable to the results of trial 1 and trial 2 in the current research.

Non-target injury to native aquatic plants is also a major concern when managing infestations of invasive weeds. Hutchinson and Langeland (2008) documented several broadleaf wetland plants were sensitive to applications of metsulfuron; however, sand chord grass (*Spartina bakeri* Merr.), soft rush (*Juncus effuses* L.), swamp lily (*Crinum americanum* L.), and buttonbush (*Cephalanthus occidentalis* L.) were more tolerant to applications ≤ 42 g a.i. ha⁻¹. Chiconela et al. (2004) reported no significant effects on torpedo grass (*Panicum repens* L.), knotgrass (*Paspalum distichum* L.), para grass [*Brachiaria mutica* (Forssk). Stapf], or softstem bulrush [*Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla] at metsulfuron rates ≤ 70 g a.i. ha⁻¹, but pickerelweed (*Pontederia cordata* L.) and arrowhead (*Sagittaria lancifolia* L.) were negatively impacted. It should be noted that the majority of giant salvinia infestations in

Louisiana and Texas are vast monotypic mats that have negatively impacted infested waterbodies for nearly 2 decades (A. Perret, personal communication, 2017). Thus, any negative impacts of metsulfuron applications to co-occurring native aquatic plant species would likely be minimal when targeting giant salvinia.

This research provides the first documentation of metsulfuron and sulfometuron efficacy on giant salvinia, and that giant salvinia is sensitive to low use rates of metsulfuron (21 g a.i. ha⁻¹) and sulfometuron (158 g a.i. ha⁻¹) and regrowth of treated plant material is minimal. Sulfometuron is classified as general use herbicide with an acute and chronic toxicity of low to very low for rats (Oral LD₅₀ > 5000 mg kg⁻¹ body weight) and a 96 h LC₅₀ (lethal concentration to kill 50% of test population) > 12.5 mg L⁻¹ in bluegill and rainbow trout (Shaner 2014). The acute and chronic toxicity of metsulfuron is classified as low to very low for rats (LD₅₀ > 5000 mg kg⁻¹) and bluegill sunfish (96 h LC₅₀ > 150 mg L⁻¹) (Bayer 2015). With regard to metsulfuron, the lower toxicity to non-target aquatic organisms and its efficacy on giant salvinia make it a more suitable potential candidate for registration as an aquatic herbicide or SLN label in Louisiana and Texas where giant salvinia infestations are continuing to spread annually. The SLN label in Florida for controlling OWCF allows applications in/on freshwater marshes (sloughs, wet prairies, and sawgrass marshes), floodplains, swamps, and Everglades tree islands (Bayer 2017b). This landscape is similar to giant salvinia infested areas of Louisiana and Texas, and hopefully a metsulfuron SLN label would be possible in both states. Alternative application techniques such as an in-water injection treatments and lower use rates ≤ 21 g a.i. ha⁻¹ should be examined in future research.

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2.5. Sources of Materials

¹ Miracle-Gro® Lawn Fertilizer, The Scotts Company, P.O. Box 606 Marysville, Ohio 43040.

² Londax®, RiceCo LLC, 5100 Poplar Avenue, Suite 2458 Memphis, TN 3817.

³ Halomax™, Aceto Agricultural Chemicals Corporation, 4 Tri Harbor Court, Port Washington, NY 11050.

⁴ Cimarron® Max part A, DuPont. E.I. du Pond de Nemours and Company, 1007 Market Street, Wilmington, DE 19898.

⁵ TranXit®, DuPont. E.I. du Pond de Nemours and Company, 1007 Market Street, Wilmington, DE 19898.

⁶ Oust® XP, DuPont. E.I. du Pond de Nemours and Company, 1007 Market Street, Wilmington, DE 19898.

⁷ Envoke®, Syngenta Crop Protection LLC. P.O. Box 18300, Greensboro, NC 27419-8300.

⁸ Sharpen®, BASF Corporation 26 Davis Drive, Research Triangle Park, NC 27709.

⁹ Resource®, Valent U.S.A. Corporation, P.O. Box 8025. Walnut Creek CA 94596-8025.

¹⁰ Command® 3ME, FMC Corporation, Agriculture Products Group, Philadelphia, PA 19103.

¹¹ Liberty®, Bayer CropScience LP, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

¹² Procellacor™ EC, SePro Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

¹³ Poast®, BASF Corporation 26 Davis Drive, Research Triangle Park, NC 27709.

¹⁴ Turbulence™, Winfield Solutions LLC, P.O. Box 64589 St. Paul, MN 55164.

¹⁵ TeeJet®, Spraying Systems Co., P.O. Box 7900 Wheaton, IL 60187.

Chapter 3. Investigating Herbicide Resistance In Giant Salvinia Populations Exposed To Multiple Glyphosate Applications Annually

3.1. Introduction

Giant salvinia (*Salvinia molesta* D.S. Mitchell) is a highly invasive floating aquatic fern that is spreading across the southern United States. Also known as “Kariba weed” or “African pyle”, giant salvinia has migrated from its native range in South America to over 20 countries worldwide (Oliver 2003). It was first reported in the United States in South Carolina in 1995 (Johnson 1995), and has since spread across the southern United States as far west as California and Hawaii (Thayer et al. 2018). Infestations lead to ecological and economic impacts including, but not limited to, decreased wildlife habitat and water quality, disruption of transportation, irrigation, and recreational activities, lowered property values, mosquito breeding habitat, and public health concerns (Jacono 1999, Jacono and Pittman 2001, Nelson et al. 2001).

Giant salvinia growth is rapid with biomass doubling in 36 hours under optimal conditions (Johnson et al. 2010). Dense mats of plant material can completely cover a water body and mats as thick as 1 meter have been reported (Thomas and Room 1986, McFarland et al. 2004). Giant salvinia has become especially problematic in Louisiana after it was first documented in Toledo Bend Reservoir in 1998 (Horst and Mapes 2000). The state is comprised of 12 major water basins that contain over 222,500 hectares of surface water (United States Census Bureau 2010). This myriad of interconnecting water resources has contributed to the spread of giant salvinia and it currently occupies water bodies throughout the majority of the state (C. Mudge, personal communication, 2018).

Physical, biological, chemical, and integrated control techniques are all utilized in Louisiana to manage giant salvinia. Chemical management using United States Environmental Protection Agency (USEPA) Section 3 registered aquatic herbicides is the most commonly used technique, and currently the Louisiana Department of Wildlife and Fisheries (LDWF) manages over 8,000 hectares of giant salvinia annually (A. Perret, personal communication, 2017). Fourteen herbicides are currently registered for use in and around aquatics systems by the USEPA (Netherland and Jones 2012, University of Florida 2014). Ten of the fourteen registered herbicides have activity on giant salvinia, including: bispyribac-sodium, carfentrazone-ethyl, copper, diquat dibromide, endothall, flumioxazin, fluridone, glyphosate, penoxsulam, and topramezone (Nelson et al. 2001, Glomski et al. 2003, Glomski and Getsinger 2006, Glomski and Mudge 2013, Mudge and Harms 2012, Mudge et al. 2012, 2013, Mudge 2016).

Contact herbicides are typically applied to the foliage (i.e. fronds) of giant salvinia. Plant injury is rapid and death has been documented three to four days after treatment (Nelson 2014) and often sooner under optimal temperatures. Unfortunately, these products do not translocate throughout the plant and only tissue that has been contacted with the herbicide will be impacted. This can be problematic in dense infestations where underlying plants are not exposed to the herbicide solution. Systemic herbicides such as glyphosate, fluridone, bispyribac-sodium, penoxsulam, and topramezone are translocated throughout the plant, but injury symptoms appear more gradual than that of contact herbicides (Nelson 2014). In addition, plants treated with subsurface applications of the systemic herbicides bispyribac-sodium, fluridone, and

penoxsulam, must be exposed to these slow-acting products for several weeks to achieve control (Mudge et al. 2012, Glomski and Mudge 2013).

In Louisiana, tank mixes of glyphosate [3.4 kg acid equivalent (a.e.) ha⁻¹] and diquat [0.56 kg active ingredient (a.i.) ha⁻¹] are administered almost exclusively for giant salvinia control (Mudge et al. 2016). Diquat is utilized to quickly desiccate the trichomes (hairs) on the frond surface, assist glyphosate uptake, and offer rapid visual markers a few hours after application to distinguish treated versus non-treated sites (Mudge and Netherland 2014). Due to the difficulty of achieving 100% herbicide coverage to each individual frond, glyphosate is beneficial because of its ability to be translocated throughout the plant (Nelson 2014). Although this herbicide combination has been a successful treatment option for many years, recent anecdotal evidence from applicators and natural resource managers has led to claims that glyphosate is not as efficacious as previously reported with plants recovering in as few as 30 days after treatment (DAT). These claims has led to concerns of developing a glyphosate-resistant giant salvinia population.

Globally, 38 plant species are resistant to glyphosate, 16 of which are present in the United States (Heap 2017). Glyphosate resistance in aquatic plants has not been documented, and herbicide resistance in aquatics has been limited to fluridone resistant hydrilla (*Hydrilla verticillata* L.f. Royle) (Michel et al. 2004) and diquat resistant landoltia duckweed [*Landoltia punctata* (G. Meyer) D.H. Les and D.J. Crawford] (Koschnick et al. 2006). Glyphosate resistance in terrestrial weed species has been linked to a weak target site mutation (target site-based resistance) and/or a reduced glyphosate translocation mechanism (non-target site-based resistance) (Powles and Preston 2006).

Weak target site mutation resistance has been linked to a mutation of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, causing a proline-to-serine substitution at amino acid 106 (Powles and Preston 2006). Reduced glyphosate translocation resistance has been documented in several populations of Australian rigid ryegrass (*Lolium rigidum* Gaudin) where it was determined that susceptible plants accumulated glyphosate in the lower plant parts and roots, whereas resistant plants accumulated glyphosate in the tip of the treated leaf with little herbicide movement into the roots (Lorraine-Colwill et al. 2002).

Although glyphosate resistant giant salvinia has not been documented, the majority of infestations throughout Louisiana and Texas have been exposed to glyphosate annually for over a decade (C. Mudge, personal communication, 2017). With a limited number of efficacious herbicides available to control this floating species, it would be detrimental if a glyphosate resistant population of giant salvinia were to develop. Therefore, the objective of this research was to evaluate glyphosate efficacy on giant salvinia collected throughout Louisiana to determine if adequate plant control can still be achieved with this herbicide.

3.2. Materials and Methods

An outdoor mesocosm trial was conducted and repeated at the Louisiana State University (LSU) AgCenter Aquaculture Research Facility in Baton Rouge, LA, to determine if glyphosate resistant giant salvinia has developed in select water bodies throughout Louisiana. Plants were collected from Lake Bisteneau, Caddo Lake, Cross Lake, Saline Lake, Turkey Creek Lake, Toledo Bend Reservoir, and the Red River

Waterway Commission Research Station (RRWCRS) in May 2017 and transferred to 1,135 L Rubbermaid stock tanks at the LSU Aquaculture Facility (Table 3.1). All populations, except the population collected from the RRWCRS (hereafter referred to as non-managed), have been intensively managed with glyphosate for over a decade, whereas the non-managed population has not been exposed to aquatic herbicide treatments.

Table 3.1. Information collected from the Louisiana Department of Wildlife and Fisheries for waterbodies with giant salvinia populations previously managed with glyphosate in Louisiana.

Population/Waterbody	First Documentation of Giant Salvinia	Years Managed	Total glyphosate (gallons) ^a
Lake Bisteneau	2006	2007 to Present	28,495
Caddo Lake	2006	2006 to Present	5,895
Cross Lake	2006	2009 to Present	8,053
Non-Managed	N/A	N/A	0
Saline Lake	2007	2007 to Present	18,317 ^b
Toledo Bend Reservoir	1998	Pre 2005 to Present	9,220 ^c
Turkey Creek Lake	2007	2007 to Present	12,720 ^d

^a Data provided by the Louisiana Department of Wildlife and Fisheries Office of Fisheries Waterbody Management Plan Series Lake History and Management Issues (LDWF 2015, 2016a, 2016b, 2017a, 2017b, 2017c)

^b Total glyphosate data not available; data shown is representative of all herbicides applied 2007 to 2015.

^c Data represents years 2006 to 2015.

^d Data represents years 2007 to 2015.

Stock populations from each waterbody were kept separate and maintained until plants reached the tertiary growth stage. On 12 June and 22 August 2017, equal amounts of fresh plant material, enough to cover approximately 85% of the water surface, was

transferred from the stock tanks and placed into 91-76 L plastic containers (49.5 cm diameter by 58.4 cm height) filled with approximately 60 L of pond water (pH 8.5). Pond water was amended with sphagnum moss (14 g) to lower the pH to < 7.0. To encourage plant growth, 2.1 g Miracle-Gro®¹ Water Soluble Lawn Food (24-8-16) was applied initially and every two weeks throughout the 8 week study. Plants were allowed 14 days to acclimate to experimental conditions prior to herbicide application. At the conclusion of the 2 week acclimation period, plants were actively growing, healthy, and had begun to form multiple layers. As a result of the overcrowding, the plant material was thinned in all tanks 2 hours prior to treatment, so that approximately 95% of the water surface was covered with plants to ensure all fronds would be exposed to the herbicide solution.

Plants from each waterbody, hereafter referred to as population, received foliar herbicide applications of glyphosate² at 3.4 kg a.e. ha⁻¹ or 4.2 kg a.e. ha⁻¹, which are rates typically used for managing giant salvinia in Louisiana and Texas, respectively. In addition, a non-treated reference for each population was included to monitor plant growth without herbicide application. All herbicide treatments included a modified vegetable oil and non-ionic organosilicon surfactant blend³ at 0.25% v/v. Foliar applications were administered using a CO₂-powered sprayer at an equivalent of 935 L ha⁻¹ diluent delivered through a single TeeJet⁴ 80-0067 nozzle at 20 psi. Each treatment was replicated 4 times for a total of 84 experimental units. In addition, seven pre-treatment biomass samples, one from each population, were collected prior to herbicide application.

At 8 weeks after treatment (WAT), all viable plant material was collected, dried to a constant weight (65° C), and recorded as grams dry weight biomass. Dry weight biomass were sorted by population and converted to percent biomass reduction of the non-treated reference within a given population using the following equation:

$$(\text{Reference Biomass} - \text{Treatment Biomass}) \div \text{Reference Biomass} * 100 = \% \text{ Biomass Reduction.}$$

Percent biomass data were pooled across both trials and subjected to an analysis of variance (ANOVA) using Proc Glimmix procedure in SAS® version 9.4 (2017) statistical software with population and rate as fixed effects and trial as a random effect. If a significant population effect or population by rate interaction were detected, means were separated using Fisher's Protected LSD test at $p \leq 0.05$ significance level. LS-means were used for all statistical comparisons, but true means are reported in text.

3.3. Results and Discussion

There was not a significant population effect ($p = 0.9411$) or population by herbicide rate interaction ($p = 0.5280$); however, there was a significant herbicide rate effect ($p = 0.0080$). When averaged across populations, the 4.2 kg a.e. ha⁻¹ rate of glyphosate provided better control than the 3.4 kg a.e. ha⁻¹ rate, resulting in 98 and 95% control, respectively (Table 3.2). Although plants were arranged as a single layer to encourage complete herbicide to frond contact, individual fronds survived in several of the test populations. Post-treatment photograph analysis of one tank, treated with the 3.4 kg a.e. ha⁻¹ rate of glyphosate, revealed that small submersed fronds were

Table 3.2. The response of various field collected populations of giant salvinia from throughout Louisiana to foliar applications of glyphosate 8 weeks after treatment.

Population/Waterbody	% Biomass Reduction	
	3.4 kg a.e. ha ^{-1a}	4.2 kg a.e. ha ⁻¹
Lake Bisteneau	95 ± 2 ^b	98 ± 2
Caddo Lake	95 ± 5	100 ± 0
Cross Lake	95 ± 3	100 ± 0
Non-Managed	96 ± 3	100 ± 0
Saline Lake	97 ± 2	100 ± 0
Toledo Bend Reservoir	95 ± 5	98 ± 1
Turkey Creek	94 ± 2	95 ± 4
Average [*]	95 ± 1	98 ± 1

^a A modified vegetable oil and non-ionic organosilicon surfactant blend at 0.25% v/v was included with all treatments.

^b Data are shown as percent of the non-treated reference of each population (mean ± SE); n = 8

^{*} Notes a significant difference between means at $p \leq 0.05$ significance level.

present during herbicide application and subsequently were not contacted with herbicide solution. Visual inspections documented 3 newly formed submersed fronds 1 WAT, 19 fronds at 2 WAT, 183 fronds at 3 WAT, and 366 fronds at 4 WAT. Consequently, the number of viable fronds increased 123 fold by 25 DAT. Because it was known that all fronds within the tank were not fully exposed to herbicide solution, it was omitted from the statistical analysis.

These results highlight the difficulty of obtaining 100% giant salvinia control, most notably in a field scenario where factors such as boat wash, wave action, wind, co-

occurring vegetation, and obstructions may hinder adequate coverage. Management programs resulting in poor plant control is likely attributed to reduced glyphosate translocation, herbicide solution wash off (i.e. boat wake), the presence of multiple plant layers, and/or missed plant material.

In reference to limited herbicide translocation, the orientation, growth, and/or nutrient allocation of giant salvinia may be limiting glyphosate movement within the plant. Each ramet is composed of a horizontal rhizome with an internode, node, two floating fronds, apical bud, auxiliary buds, and a submersed frond that acts and appears as a root (Room 1983). As a result, glyphosate translocation within a giant salvinia rhizome would need to be vertical and horizontal, as opposed to just vertical, which is typical of most other plant species. Although the parent plant was exposed to the herbicide solution, lethal amounts of glyphosate may not have translocated to newly formed fronds and/or buds below the water surface.

The translocation characteristics of a given herbicide can vary among plant species (Hutchinson et al. 2010) and there is limited information referencing herbicide translocation within aquatic ferns. Bowmer et al. (1993) and Tucker et al. (1994) reported limited translocation of glyphosate to roots and new rhizomes in terrestrial alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb]. Similarly, Hutchinson et al. (2010) evaluated foliar spray, cut-and-spray, and basal applications of glyphosate and triclopyr to old world climbing fern [*Lygodium microphyllum* (Cav.) R. Br. OWCF] and observed relatively no basipetal movement into the rhizome with 100% foliar spray applications; however, some basipetal movement into the rhizomes was observed with

cut and spray and basal applications. It was also documented that no data supported the horizontal movement of either herbicide along OWCF rhizomes (Hutchinson et al. 2010).

Although glyphosate is registered as an aquatic herbicide for foliar use, it is inactive once it reaches the water column, which is likely attributed to sediment adsorption and microbial degradation (Brønstad and Friestad 1985). Herbicide movement from foliage to submersed fronds (i.e. roots) or rhizomes in emergent and floating aquatic plants may be limited under deep water or flooded conditions. Research has reported limited or no translocation of 2,4-D from leaves and stems of cattail [*Typha angustifolia* L. var. *brownii* (Kunth) Kronf.] to horizontal rhizomes under flooded conditions (Levi 1960). In addition, Mudge and Netherland (unpublished data) documented glyphosate alone and in combination with carfentrazone-ethyl or flumioxazin provided better control of torpedo grass (*Panicum repens* L.) under non-flooded conditions when the entire stem was exposed compared to flooded conditions.

Since fragmentation is the only verified means of giant salvinia reproduction, it could be speculated that each pair of submersed and adjacent/corresponding emergent fronds act independently in acquiring nutrients. Thus, the majority of giant salvinia nutrient transport may occur between submersed fronds and the emergent fronds directly above them. This autonomous self-sufficient system would ensure plant fragments are able to maintain active growth and spread to new areas through colonization. In contrast, visual observations in the current research and field settings have shown herbicide injury to be present in developing immature fronds attached to adjacent previously treated mature fronds. Theoretically, newly formed fronds may rely on mature adjacent fronds for nutrient allocation until a self-sufficient functional group of submersed fronds are

formed. These factors may be responsible for rapid plant recovery if mature plants with fully developed submersed fronds are not directly contacted by glyphosate.

Results from this research suggest that giant salvinia populations that have been managed annually with glyphosate are no less susceptible than those where glyphosate has not been applied. Sub-optimal control and plant recovery in field sites is likely due to limited herbicide coverage, herbicide wash off, and/or limited herbicide translocation to submersed fronds. However, it should be noted that the plants used in the current study only represent a small sub-set of giant salvinia within each population and it could be speculated that a resistant plant(s) may be present within one or more of these waterbodies. Unfortunately, detection will be difficult until selection pressure of repeated glyphosate applications allows these plants to become more pronounced within a population. Incorporating herbicides with different modes of action in to a management plan, which lowers the selection pressure of a particular weed species, is a prime strategy for managing herbicide resistance.

Evolution of herbicide resistance in higher plants is considered unlikely in the absence of sexual reproduction (Hill 1982, Maxwell and Mortimer 1994); however, both aquatic species with previously documented herbicide resistance - fluridone resistant hydrilla (Michel et al. 2004) and diquat resistant landoltia duckweed (Koschnick et al. 2006) - reproduce asexually and spread by fragmentation. Although neither of these species are resistant to glyphosate, the development of glyphosate resistant giant salvinia could follow a similar trend. Consequently, weed management programs often do not change until herbicide resistance has already occurred (Beckie 2006). Low adoption of herbicide rotation may be due to a lack of available efficacious products (Beckie 2006),

particularly in aquatics where a limited number of active ingredients are available.

However, recent herbicide screening trials with registered non-aquatic herbicides have yielded promising results, which could subsequently lead to the aquatic labeling of additional herbicides for managing giant salvinia.

Future research should examine the translocation of herbicides commonly used for managing giant salvinia to determine if results vary among populations. In addition, diquat efficacy should also be investigated due to its annual use as a tank mix partner and sole use during the winter. Lastly, maintaining thorough treatment records and visual evaluations of managed sites will be beneficial for early detection and rapid response if an herbicide resistant giant salvinia population were to be confirmed.

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3.5. Sources of Materials

¹ Miracle Gro® All Purpose Plant Food, The Scotts Company LLC, P.O. Box 606 Marysville, Ohio 43040.

² Roundup Custom™, Monsanto Company, 800 N. Lindbergh Blvd, St. Louis, MO 63167.

³ Turbulence™, Winfield Solutions LLC, P.O. Box 64589 St. Paul, MN 55164.

⁴ TeeJet®, Spraying Systems Co., P.O. Box 7900 Wheaton, IL 60187.

Chapter 4. Giant Salvinia Response to Foliar Herbicide Applications During Winter

4.1. Introduction

The first reported documentation of giant salvinia (*Salvinia molesta* D.S. Mitchell) in the United States occurred in 1995 in South Carolina (Johnson 1995). Since its introduction, it has spread across the southern United States and as far west as California and Hawaii (Thayer et al. 2018). Giant salvinia was first documented in Louisiana in 1998 on the Toledo Bend Reservoir, which lies on the Louisiana-Texas border (Horst and Mapes 2000), and has since dispersed across Louisiana and East Texas. These infestations have caused ecological and economic impacts including, but not limited to, reduction of desirable native plant species, disruption of transportation and irrigation, decreased recreational use, and increased mosquito breeding habitat (Jacono 1999, Jacono and Pittman 2001, Nelson et al. 2001). These negative impacts have led to active management operations for controlling giant salvinia in Louisiana and Texas.

Small scale research and large-scale field operations have shown that herbicides registered for aquatic use in the United States are capable of managing giant salvinia infestations (McFarland et al. 2004), but its growth habits make it difficult to get adequate control with foliar herbicide applications. Rapid growth and the ability to quickly form multiple plant layers often prevents foliar applied herbicides from reaching fronds and plants that are found under the upper mat layers (Horst and Mapes 2000). Chemical management in Louisiana is primarily administered using a combination of the aquatic herbicides glyphosate + diquat and two adjuvants during the growing season from April through October (Mudge et al. 2014, Mudge et al. 2016). In 2016, the Louisiana

Department of Wildlife and Fisheries (LDWF) chemically managed > 8,000 hectares of giant salvinia in public waterbodies throughout the state (A. Perret, personal communication, 2017).

Well established giant salvinia infestations are typically multiple plant layers thick and require multiple herbicide applications throughout the year to achieve adequate control (Mudge et al. 2016). The winter months may be an ideal time for natural resource managers to utilize foliar applied herbicides to achieve favorable control. During periods of cold weather, multiple plant layers are often not present, plant biomass is substantially reduced, and as a result, fewer herbicide applications would be required when plant growth is much slower compared to the spring, summer, and fall. Winter applications may also allow herbicides to contact a larger portion of the target plant population since one layer of plant material is likely present during December, January, and February.

Many natural resource agencies speculate that herbicides are not effective for managing aquatic plants during the winter because plants are slow growing or dormant due to lower temperatures and shorter photoperiods (Mudge and Sartain 2018). Applications of diquat [1.7 kg active ingredient (a.i.) ha⁻¹] + one surfactant are sometimes used to manage severe giant salvinia infestations during winter in Louisiana (Mudge et al. 2014). In addition, Federal and state agencies in Texas have managed giant salvinia with glyphosate + diquat treatments during winter (T. Corbett, personal communication, 2017). However, a limited number of replicated studies have been conducted to evaluate herbicide efficacy on giant salvinia during the winter months (Mudge and Sartain 2018) and replicated research investigating standalone and/or combination treatments at multiple winter timings have not been conducted. To investigate herbicide efficacy on

giant salvinia at various winter application timings the following mesocosm research was conducted.

4.2. Materials and Methods

Outdoor mesocosm trials were conducted at the Louisiana State University (LSU) AgCenter Aquaculture Research Facility in Baton Rouge, LA, to investigate herbicide efficacy against mature giant salvinia during the winter of 2015-2016 (initial) and 2016-2017 (repeat). Tertiary growth-stage plants collected from stock tanks at LSU Aquaculture were cultured in 90 (76 L) plastic containers (49.5 cm diameter by 58.4 cm height) filled with approximately 60 L of pond water (pH 8.5) amended with sphagnum moss (14 g) to lower the pH to < 7.0. On 18 September 2015 and 29 September 2016, equal amounts of fresh plant material, enough to cover approximately 85% of the water surface, were placed in each 76 L tank. In addition, 2.1 g of Miracle-Gro®¹ Water Soluble Lawn Food (24-8-16) was applied to each container at planting and every 2 weeks until mid-December. Fertilization resumed in mid-March and continued every 2 weeks in both trials until final harvest. Culture and planting techniques were adapted from previous giant salvinia research (Nelson et al. 2007, Mudge et al. 2012, Mudge et al. 2016).

Plants received foliar herbicide applications at one of the three application timings. Treatments during the initial trial, hereafter referred to as trial 1, were applied 12 December 2015, 12 January 2016, and 11 February 2016. Treatments during the repeat trial, hereafter referred to as trial 2, were applied 6 December 2016, 4 January 2017, and 6 February 2017. These timings were chosen to compare early, mid, and late

winter applications. Each treatment was replicated five times for each application timing. Non-treated reference plants were used to compare plant growth in the absence of herbicide. Hourly air temperature data during both trials were obtained from a local weather station (LSU AgCenter 2017).

Prior to the December treatment, the plant canopy was several layers thick (> 5 cm). Herbicide treatments were administered using a CO₂-powered sprayer at an equivalent of 935 L ha⁻¹ diluent delivered through a single TeeJet®² 80-0067 nozzle at 20 psi. Herbicide treatments included: diquat³ (1.1 kg a.i. ha⁻¹), glyphosate⁴ (3.4 kg acid equivalent (a.e.) ha⁻¹), flumioxazin⁵ (0.2 kg a.i. ha⁻¹), glyphosate (3.4 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹), and a non-treated control. All herbicide treatments contained a combination of a non-ionic organosilicon surfactant⁶ (0.1% v/v) and a non-ionic surfactant⁷ (0.25% v/v). In addition, 5 pre-treatment samples were collected prior to each application timing to quantify plant biomass. Final plant harvest was conducted twelve weeks post February treatment. On 9 May 2016 and 1 May 2017, viable plant material were collected, dried to a constant weight (65° C), and recorded as grams dry weight biomass. Biomass measurements were converted to percent biomass reduction of the non-treated reference within each application timing (i.e. December, January, or February) using the following equation: (Reference Biomass - Treatment Biomass) ÷ Reference biomass * 100 = % Biomass Reduction.

Due to varying winter conditions between trial 1 and trial 2 (i.e. plants were exposed to colder conditions in trial 2), percent biomass data were separated by year and subjected to an analysis of variance (ANOVA) with herbicide treatment and application timing as fixed effects. Type III tests were used to test for significant fixed effects. LS-

means were used for treatment and timing comparisons with means separated using a Fisher's Protected LSD method. All statistical analysis were conducted in SAS® version 9.4 (2017) statistical software at $p \leq 0.05$ significance level.

4.3. Results and Discussion

With regard to biomass reduction 12 weeks after the February application, there was no significant herbicide treatment by application timing interaction in trial 1 ($p \leq 0.06476$) or trial 2 ($p \leq 0.4757$). However, significant differences were noted for herbicide treatment and application timing in trial 1 (Table 4.1). Diquat, flumioxazin, glyphosate, and glyphosate + diquat treatments, pooled across application timing, reduced giant salvinia biomass by 27, 45, 55, and 55%, respectively. Applications during January and February resulted in greater control compared with December (47 and 50% vs. 33%, respectively). In trial 2, giant salvinia control was $\geq 99\%$ and herbicide treatment ($p = 0.3818$) and application timing ($p = 0.4007$) were not significant.

4.3.1. Trial 1

In trial 1, December applications of diquat, flumioxazin, and glyphosate + diquat resulted in plant injury less than 1 week after treatment (WAT). As expected, glyphosate injury was slower to develop and injury symptoms did not become visible until 3 WAT. Although, diquat and flumioxazin treatments resulted in injury less than 5 days after treatment (DAT), plant recovery was observed by 2 WAT. Visually, the upper layers of plant material appeared necrotic and non-viable; however, the necrotic plant tissue on the surface sheltered healthy actively growing fronds underneath the upper plant layer.

Table 4.1. The response of giant salvinia (LS-mean percent control \pm SE) to the aquatic herbicides diquat, flumioxazin, glyphosate, and glyphosate + diquat, applied during the winter trial 1 and trial 2.

Treatment Factor ^a		Giant Salvinia % Biomass Reduction	
Herbicide ^{b,c}	Rate (kg a.i. ha ⁻¹) ^d	Trial 1	Trial 2
Diquat	1.1	27 \pm 4 a ^e	99 \pm 0 ns
Flumioxazin	0.2	45 \pm 4 b	100 \pm 0 ns
Glyphosate	3.4	55 \pm 4 b	100 \pm 0 ns
Glyphosate + Diquat	3.4 + 0.5	45 \pm 4 b	100 \pm 0 ns
Application Timing			
December		33 \pm 4 a	100 \pm 0 ns
January		47 \pm 4 b	100 \pm 0 ns
February		50 \pm 3 b	100 \pm 0 ns

^a Data for each treatment factor are pooled over all levels of the other factor. Means within a column for each treatment factor followed by the same letter are not significantly different according to Fisher's Protected LSD test $p \leq 0.05$.

^b Diquat, flumioxazin, glyphosate, and glyphosate + diquat applied at 1.1, 0.2, 3.4, and 3.4 + 0.5 kg a.i. ha⁻¹, respectively.

^c A non-ionic organosilicone surfactant + non-ionic surfactant was included with each treatment at 0.1% and 0.25% (v/v), respectively.

^d Glyphosate applied as kg a.e. ha⁻¹

^e Data are normalized to the non-treated reference during each respective winter.

This sheltering effect was also observed by Mudge and Sartain (2018) who reported winter treatments were less efficacious to giant salvinia protected from cold air temperatures when compared to exposed plants. In addition, Mudge et al. (2016) documented substantially less giant salvinia control with glyphosate + diquat and endothall + flumioxazin during the fall when plant growth is slower compared to these same treatments applied during the spring and summer months.

Treatments applied in January and February of trial 1 were more effective than applications in December. Plant material was subjected to freezing temperatures prior to herbicide applications in January and February (Figure 4.1). Unfortunately, giant salvinia

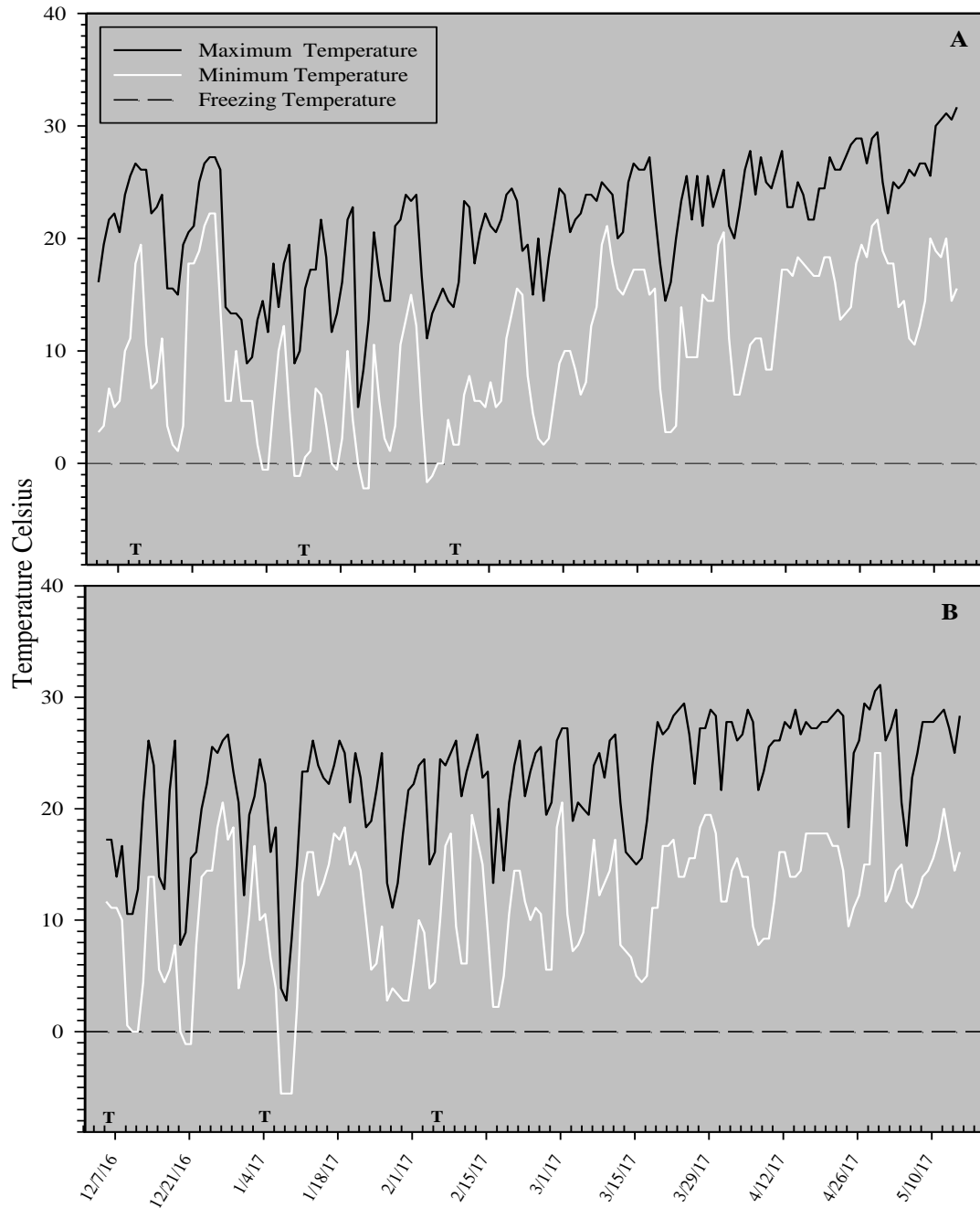


Figure 4.1. Maximum and minimum daily air temperatures ($^{\circ}\text{C}$) A) 3 December 2015 to 20 May 2016 and B) 3 December 2016 to 20 May 2017. The symbol “T” along the x-axis represents the date of the herbicide application

buds are capable of tolerating infrequent frosts and freezes (Whiteman and Room 1991). Larger fronds can provide protection from cold air temperatures, thus allowing viable buds to persist (Harley and Mitchell 1981). Individual fronds or whole plants exposed to heavy frosts or sub-freezing temperatures could die off or be substantially reduced in biomass, but water and upper portions of the plant mat can insulate stems and lateral buds that protect the plant during unfavorable conditions and ultimately lead to recovery in the spring (Owens et al. 2004). Cold exposure studies have documented that the average water temperature is likely to be 1.4, 1.6, and 1.8 times greater in low, medium, and high density giant salvinia biomass treatments, respectively, compared to open water treatments when plants were exposed to 0° C for 14 hours (Moshman 2018).

Minor freeze and frost related injury symptoms from cold exposure were noted prior to the January treatment in trial 1. Weather related injury symptoms were more severe in February as observed on control plants, and pre-treatment biomass samples indicated that dry weight biomass decreased slightly from 53.4 ± 2.5 g in January to 52.7 ± 3.4 g in February (Figure 4.2). Trial 1 reference plants displayed freeze damage in 95% of the emergent fronds at February treatment. The excessive amount of freeze damaged fronds may have led to decreased diquat efficacy at the February application timing. Diquat applications in January provided 45% control compared to only 20% in February applications (data not shown). Although the plant biomass was slightly less in February, approximately 5% of the upper emergent fronds were healthy with green tissue present. Since diquat is not actively translocated throughout the plant, it is less effective when it is not able to contact healthy plant material or applied to injured/non-viable tissue, which was the case in February 2016 for trial 1. These data are not supportive of

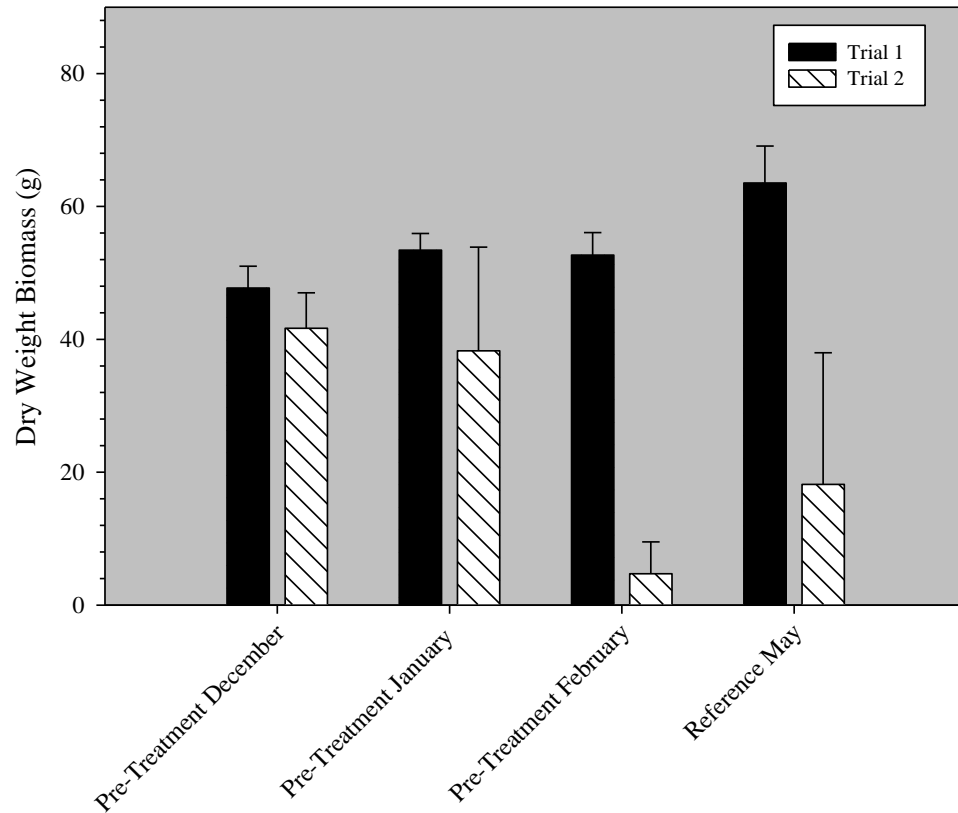


Figure 4.2. Dry weight biomass (mean \pm SE) of giant salvinia harvested pre-treatment and from non-treated reference during the winter for trial 1 (2015-2016) and trial 2 (2016-2017).

previous research by Mudge and Sartain (2018) that documented diquat provided $\geq 93\%$ control of giant salvinia when applied during the winter; however, treatments were administered earlier in the winter prior to cold weather inception. Most likely differences in harvest date and plant architecture (single layer vs. multiple layers of plant material) contributed to these differences between the current and previous research (Mudge and Sartain 2018).

4.3.2. Trial 2

All December treatments in trial 2 resulted in $\geq 10\%$ visual injury to giant salvinia when compared to the reference at 1 WAT. The rapid visual injury symptoms observed

on plants treated with glyphosate was unexpected since this treatment produced substantially slower injury when applied to giant salvinia in trial 1 of the previous year. Plants in trial 2 were exposed to freezing temperatures 5 days post December application, which may have contributed to the faster visual injury. It has been documented in several terrestrial plant species that glyphosate efficacy increases as temperature increases (Adkins et al. 1998; Waltz et al. 2004), however these data have been inconsistent with other findings. Zhou et al. (2007) documented that glyphosate treated velvetleaf (*Abutilon theophrasti* Medik.) exposed to post-treatment temperatures of 5° and 12° C for 48 hours enhanced glyphosate control of velvetleaf, but cold stress prior to treatment decreased glyphosate efficacy. Reddy (2000) reported that absorption and translocation of glyphosate in redvine [*Brunnichia ovata* (Walt.) Shinnery] was higher in plants maintained at 15/10° C than 25/20° C (day/night). The variable results of temperature effects on the translocation and absorption of glyphosate is most likely due to different bioassay species and temperature regimes (Zhou et al. 2007). December treated plants had been exposed to multiple minor freeze events at 2 WAT (Figure 4.1) and by 4 WAT were reduced to a single layer of plant material. Although all plants were exposed to minor freezes, only the herbicide treated plants were reduced to a single plant layer, whereas the reference plants and those that were yet to be treated (i.e. January and February treatments) still maintained multiple layers of plant material.

Four days post January treatment in trial 2, a prolonged freezing period occurred. During this time, air temperatures remained at or below freezing (as low as -6° C) for 33 hours over a 38 hour period (Figure 4.1). This resulted in ice formation up to 8 cm thick in the experimental containers. The effects of the prolonged freeze period were

immediately evident. Pre-treatment biomass decreased 87% from January 2017 to February 2017 and plant material that had not been treated was reduced to a single layer. The response of giant salvinia to the prolonged freeze is in agreement with data generated by Whiteman and Room (1991) that documented temperatures below -3°C can be lethal to giant salvinia buds if exposure exceeds 2 hours. Although freezing temperatures can have lethal effects on giant salvinia and/or decrease the number of plants to a remnant population, regrowth will often occur from underlying insulated plants or from plants not subjected to pro-longed periods of sub-freezing temperatures (Horst and Mapes 2000, McFarland et al. 2004). An acute-exposure study, where giant salvinia was exposed to low temperatures (4°C , -3°C , and -16°C) for various exposure times (1, 4, 8, 15, 24, and 48 hr), documented that exposure to -16°C for 48 hr led to complete mortality, while the other treatments failed to provide complete control (Owens et al. 2004). In the current study, the prolonged freeze period did not provide complete control of the non-treated plant material. However, biomass were reduced to a single plant layer which allowed for excellent herbicide coverage to minimal remaining biomass during February treatments. Plant response in trial 1 and trial 2 varied significantly due to dissimilar environmental conditions. Air temperatures from 5 December to 15 May were relatively mild during both winters. The number of days with air temperatures at or below freezing was 13 days in trial 1 and 8 days in trial 2. Although trial 1 had more days at or below freezing in comparison to trial 2, plants were only exposed to 27 total hours of temperatures at or below freezing compared to 45 hours in trial 2 (Table 4.2).

These data provide evidence that successful control of giant salvinia in the winter is possible; however, control will be more dependent on environmental conditions and

influenced by herbicide application timing relative to freeze/frost events. Overall, both trials provided some level of control when compared to non-treated plant material. Therefore, efforts to chemically manage giant salvinia should continue beyond the growing season into mid to early winter and/or begin in late winter as opposed to early or mid-spring. Future research should evaluate other herbicide chemistries, application rates, and temperature regimes when managing giant salvinia to more accurately predict the level of control.

Table 4.2. Average air temperature ($^{\circ}\text{C} \pm \text{SD}$) giant salvinia was exposed to during each month of the winter trial 1 (2015-2016) and winter trial 2 (2016-2017).

Month	Trial 1	Trial 2
	12/3/2015 to 5/20/2016	12/3/2016 to 5/20/2017
	Average Temperature ^a	
December	15.7 \pm 6.3	13.7 \pm 6.4
January	9.7 \pm 5.4	14.2 \pm 6.9
February	12.9 \pm 5.8	16.7 \pm 5.3
March	18.1 \pm 4.6	18.3 \pm 5.3
April	20.1 \pm 4.3	20.9 \pm 4.8
Range	-2.2 to 29.4 $^{\circ}$ C	-5.6 to 30.6 $^{\circ}$ C
Freezing Hours ^b	27	45

^a Air temperature data was collected from a local weather station operated by the LSU AgCenter in Baton Rouge, LA.

^b Number of hours air temperature was at or below 0 $^{\circ}$ C throughout the duration of each trial.

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4.5. Sources of Materials

- ¹ Miracle-Gro® Lawn Fertilizer, The Scotts Company, P.O. Box 606 Marysville, Ohio 43040.
- ² TeeJet®, Spraying Systems Co., P.O. Box 7900 Wheaton, IL 60187.
- ³ Tribune™, Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 24719.
- ⁴ Roundup Custom™, Monsanto Company, 800 N. Lindbergh Blvd, St. Louis, MO 63167.
- ⁵ Clipper™, Valent USA Corporation, P.O. Box 8025 Walnut Creek, CA 94596.
- ⁶ AirCover™, Winfield Solutions LLC, P.O. Box 64589 St. Paul, MN 55164.
- ⁷ Aqua-King Plus®, Winfield Solutions LLC, P.O. Box 64589 St. Paul, MN 55164.

Chapter 5. Effect of Winter Herbicide Applications on Bald Cypress [*Taxodium distichum* (L.) Rich.] and Giant Salvinia (*Salvinia molesta* D.S. Mitchell)

5.1. Introduction

The invasive aquatic fern giant salvinia (*Salvinia molesta* D.S. Mitchell) has continued to spread across the United States since it was first documented in 1995 in South Carolina (Johnson 1995). It exhibits very rapid growth and can double in biomass in as little as 36 hours under optimal conditions (Johnson et al. 2010). It has the ability to form large extensive mats, sometimes up to a meter thick (Thomas and Room 1986, McFarland et al. 2004), causing water resources to be negatively affected immediately after infestation. Dense giant salvinia growth impedes navigation, irrigation, and recreational use of infested water bodies (Pimentel et al. 1999) leading not only to environmental impacts, but economic impacts and public health concerns as well (McFarland et al. 2004). These negative impacts have led to situations where giant salvinia needs to be intensively managed to limit its growth and spread to surrounding water bodies.

A significant portion of the giant salvinia infestations throughout the southeastern United States occur in lakes, bayous, and backwaters that are filled with bald cypress [*Taxodium distichum* (L.) Rich.]. Bald cypress is a slow growing long lived tree species, and due to its ability to withstand prolonged flooding, it can be found in lakes, ponds, swamps, and other bottomland or water-logged areas (Samuelson and Hogan 2003). Cypress swamps are highly valued because of their ecological and economic importance, particularly their support of habitat for water fowl, wading birds, small mammals, and

other wildlife species (Samuelson and Hogan 2003). In addition, cypress swamps slow flood waters, act as sediment and pollution traps, and provide storm surge protection in areas susceptible to hurricanes and other severe weather events (Liu et al. 2009, Wharton 1977). Unfortunately, bald cypress swamps are not readily regenerating and are converting to marsh and open water (Myers et al. 1995). Poor regeneration and a loss of cypress swamps, particularly in Louisiana, is the result of altered hydrologic regimes, increased flooding, increased salinity, and other physical/biological factors (Myers et al. 1995).

Consequently, giant salvinia is frequently found growing underneath the canopy of extensive bald cypress stands, thus making it difficult for herbicide applicators to access entire plant populations. Vegetation control programs that negatively impact bald cypress are discouraged and often avoided at all costs. The difficulty of herbicide applicator crews to access these isolated areas, as well as, avoiding direct contact of herbicides to tree foliage and other tree extremities provides considerable amounts of non-managed plant material capable of rapidly re-infesting previously treated sites, making management efforts null and void.

Unlike most trees in the Cupressaceae family, bald cypress is deciduous and sheds its leaves during the winter. The annual shedding of cypress leaves may allow herbicides to be applied uniformly to giant salvinia that would otherwise be shaded during the growing season by dense bald cypress canopies. Fixed wing airplanes and helicopters are potential management tools that could be used in these situations to provide adequate herbicide coverage to areas inaccessible to watercrafts. It is unknown if aquatic herbicide applications over the top of bald cypress during the winter would provide successful giant

salvinia control with minimal or no injury to bald cypress. Since the tree is a valued commodity throughout the southeastern United States, it is too risky to attempt a large scale winter time herbicide application in a field scenario that may have irreversible negative impacts towards the health of bald cypress trees. Mesocosm trials were conducted to investigate the effects of herbicide applied over-the-top of immature bald cypress trees when foliage is minimal or completely shed, for control of co-occurring giant salvinia.

5.2. Materials and Methods

On 5 October 2015 and 25 October 2016, bald cypress trees (1.5 to 3 meters in height) were purchased from a local nursery and maintained in 58 L planting pots at the Louisiana State University (LSU) AgCenter Aquaculture Research Facility in Baton Rouge, LA. The potted trees were placed into 64 L secondary containers to prevent soil water loss through the bottom of the pots. Giant salvinia was collected from a stock population that was maintained at LSU Aquaculture and cultured in 76 L (49.5 cm diameter by 58.4 cm height) plastic containers filled with approximately 60 L of pond water (pH 8.5). Pond water was amended with sphagnum moss (14 g) to lower the pH to < 7.0. In addition, 2.1 g of Miracle-Gro®¹ Water Soluble Lawn Food (24-8-16) was applied to each container at planting and every 2 weeks until mid-December.

Fertilization resumed in mid-March and continued every 2 weeks until final harvest.

Each container of giant salvinia and potted cypress tree was randomly assigned to a respective treatment and grouped into rows of 5 alternating trees and 5 containers of giant salvinia. Five herbicide treatments included: diquat² [1.6 kg active ingredient (a.i.)

ha⁻¹], flumioxazin³ (0.2 kg a.i. ha⁻¹), glyphosate⁴ [3.4 kg acid equivalent (a.e.) ha⁻¹], and glyphosate (3.4 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹). Herbicide treatments were applied at three application timings (December, January, or February) to represent early, mid, or late winter herbicide applications. Each herbicide treatment was replicated five times at each of the three application timings. All herbicide treatments contained a combination of a non-ionic organosilicon surfactant⁵ (0.1% v/v) and non-ionic surfactant⁶ (0.25% v/v). A non-treated reference was also included for bald cypress and giant salvinia to evaluate plant health throughout the duration of the study.

In the first trial, applications were made on 15 December 2015, 13 January 2016, and 12 February 2016; and for the second trial on 14 December 2016, 12 January 2017, and 14 February 2017 over-the-top of bald cypress trees and giant salvinia. Pre-treatment giant salvinia biomass collected one hour prior to the December treatments was 38.45 g dry weight biomass and plants were in the tertiary growth stage and arranged as a single plant layer across the water surface. A CO₂-powered sprayer and custom built spray boom utilizing two TeeJet®⁷ 80-02vs nozzles at 40 psi was used. Treatments were applied at an equivalent of 94 L ha⁻¹ to simulate an aerial aquatic herbicide application at approximately 0.25 and 2.25 m above the tree and giant salvinia canopies, respectively. While administering treatments, an artificial buffer constructed from tarp and PVC pipe was used to minimize herbicide drift to adjacent treatments. In order to quantify giant salvinia health at treatment, an additional pre-treatment tank was included for each treatment and harvested prior to herbicide application.

To evaluate bald cypress injury, leaf length was assessed weekly at the first sign of bud break (March) until leaf growth slowed considerably (July). Non-destructive leaf

measurements included five leaf length measurements per branch on three pre-determined and marked branches that were at least 30 cm in length for a total of 15 leaf measurements per tree. Selected branches represented the fifth branch from the base of the tree, the fifth branch from top of the tree, and a randomly selected branch between the upper and lower selected branches. In addition, bald cypress re-foliation assessment (i.e. the production of leaves following herbicide applications) was assessed every 5 weeks on a 1 to 5 scale where 1 = equivalent to the reference, 2 = slight malformation, 3 = moderate malformation, 4 = moderate to severe malformation, and 5 = severe malformation. Re-foliation assessment compared leaf spacing, leaf length variability, leaf orientation, and overall visual aesthetics of treated trees compared to reference trees.

Giant salvinia harvest of all viable plant material for each application timing was made 12 weeks post February application. This harvest date was chosen to measure plant response well into the growing season to evaluate long term effects. Harvested giant salvinia plant material was dried to a constant weight (65° C) and recorded as grams dry weight biomass. Percent biomass reduction was calculated in relation to the non-treated reference using the following equation: $(\text{Reference Biomass} - \text{Treatment Biomass}) \div \text{Reference biomass} * 100 = \% \text{ Biomass Reduction}$.

Leaf measurement data, collected at 20 weeks after bud break (WABB), were analyzed using Proc Glimmix in SAS® version 9.4 (2017) statistical software at $p \leq 0.05$ significance level with reps and years designated as a random variable. Fixed effects included: treatment, application timing, and branch location. If significant, means were further separated using the Fisher's Protected LSD method ($p \leq 0.05$). Tree re-foliation uniformity and giant salvinia data were analyzed using the same multivariate test and

mean separation procedures. In order to provide a visual representation of leaf growth throughout the duration of the study, mean leaf length of bald cypress recorded during each data collection period were averaged across treatment, plotted, and subjected to an exponential growth to maximum non-linear regression [$y = a(1 - e^{-bx})$] in SigmaPlot®⁸ version 11.0 software.

5.3. Results and Discussion

5.3.1. Bald Cypress

Analysis of variance of bald cypress average leaf length revealed a significant effect for herbicide, timing, branch location, and treatment by timing interaction 20 WABB (Table 5.1). In addition, uniform re-foliation ratings documented a

Table 5.1. Analysis of Variance for mean leaf length (cm) of bald cypress and percent biomass reduction of giant salvinia in response to over the top applications of the aquatic herbicide diquat (1.6 kg a.i. ha⁻¹), flumioxazin (0.2 kg a.i. ha⁻¹), glyphosate (3.4 kg a.e. ha⁻¹), or glyphosate (3.4 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) in December, January, or February application timings during the winter of 2015-2016 and 2016-2017.

Parameter	F value	df	P
Bald Cypress Leaf Length (cm)			
Treatment	24.52	3, 48	< 0.0001
Time	3.94	2, 48	0.0228
Treatment x Time	4.60	6, 48	0.0006
Branch Location	8.26	2, 96	0.0005
Giant Salvinia % Biomass Reduction			
Treatment	1.48	3, 97	0.2243
Time	4.71	2, 97	0.0111
Treatment x Time	6.15	6, 97	< 0.0001

significant treatment by timing interaction ($p < 0.0001$). Averaged across all timings, leaf length for non-treated and flumioxazin treated bald cypress did not differ at 20 WABB and documented the highest average leaf length of 7.07 cm and 6.18 cm, respectively (Figure 5.1). In response to glyphosate + diquat treatments, mean leaf length (5.37 cm) was significantly less in comparison to non-treated trees, but not significantly different from flumioxazin treated trees. Bald cypress subjected to glyphosate alone and diquat alone produced the smallest average leaf length of 4.18 cm and 3.13 cm, respectively. Tree mortality was not documented in any treatment throughout the study period.

Analysis of the treatment by timing interaction revealed that trees exposed to flumioxazin at any of the three application timings did not result in a significant decrease in average leaf length when compared to reference trees 20 WABB (Figure 5.2). Leaves of non-treated and flumioxazin treated trees grew an average of 1 cm per week up to 7 WABB. After 7 WABB, leaf length increased slowly throughout the remainder of the observation period. In addition, no visual injury symptoms were recorded for flumioxazin treated trees. These results are consistent with Osieka and Minogue (2012) who reported no defoliation or malformed foliation following flumioxazin applications of 290 and 490 g a.i. ha⁻¹ to dormant bald cypress seedlings. Flumioxazin applications also resulted in the highest percentage of healthy seedlings when compared to over the top applications of sulfometuron, imazapyr, hexazinone, and aminopyralid to dormant bald cypress seedlings (Osieka and Minogue 2012). Although there is limited data on bald cypress response to flumioxazin, other conifer species have been documented as flumioxazin tolerant. Kuhns and Harpster (2002) reported Fraser fir [*Abies fraseri* (Pursh) Poir.] and Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings were not

affected by over the top spring applications of flumioxazin at 850 g a.i. ha⁻¹, which is approximately four times greater than the rate tested in the current study. Flumioxazin

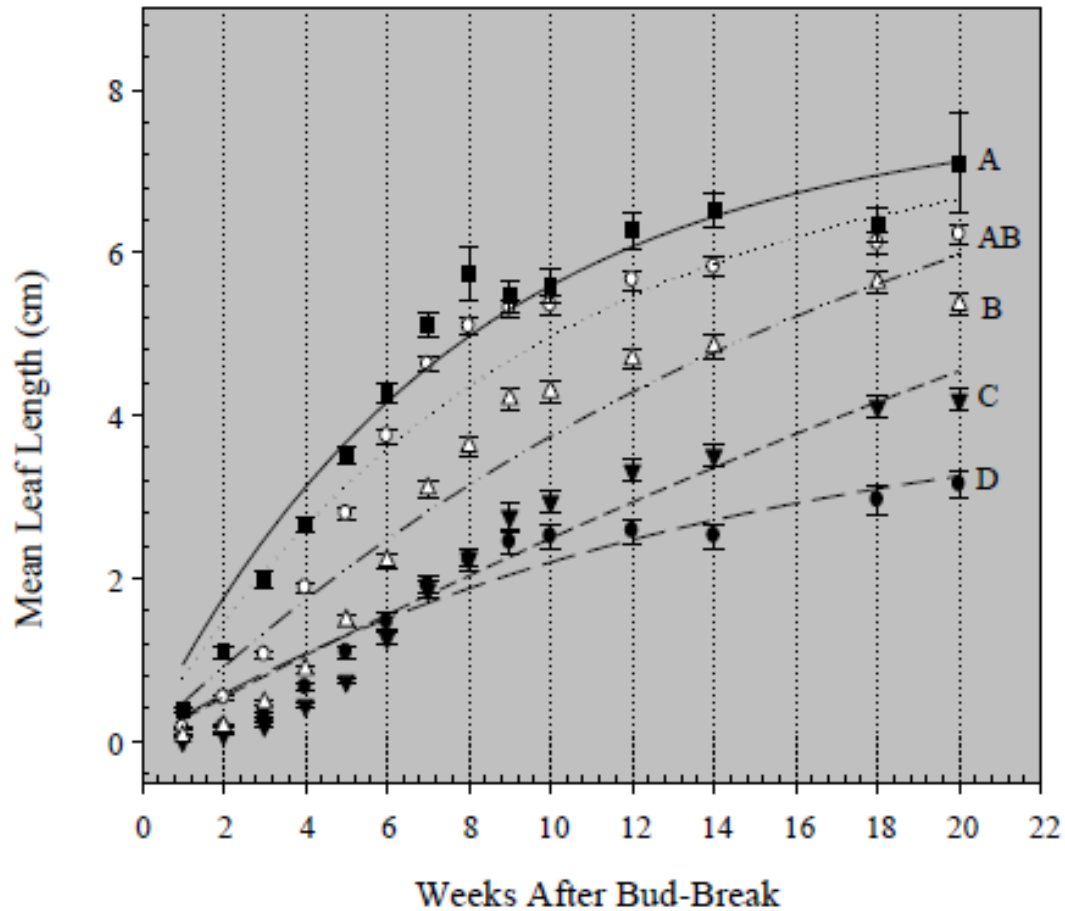


Figure 5.1. Mean leaf length of bald cypress, averaged across timing, in response to the aquatic herbicides ● diquat (1.6 kg a.i. ha⁻¹), ○ flumioxazin (0.2 kg a.i. ha⁻¹), ▼ glyphosate (3.4 kg a.e. ha⁻¹), Δ glyphosate (3.4 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) and ■ reference treatments. All measurements are reported as mean leaf length (± SE). Leaf growth was estimated using non-linear regression (Exponential growth rise to a maximum) for the diquat ($y = 4.236 \cdot 1 - e^{-0.0731x}$; $r^2 = 0.917$), flumioxazin ($y = 7.530 \cdot 1 - e^{-0.1077x}$; $r^2 = 0.921$), glyphosate ($y = 1.424 \cdot 1 - e^{-0.0192x}$; $r^2 = 0.9270$), glyphosate + diquat ($y = 9.236 \cdot 1 - e^{-0.0519x}$; $r^2 = 0.9171$), and reference ($y = 7.692 \cdot 1 - e^{-0.1298x}$; $r^2 = 0.9583$) treatments. Different letters indicate a significant mean leaf length difference at 20 weeks after bud break according to Fisher's Protected LSD test ($p \leq 0.05$).

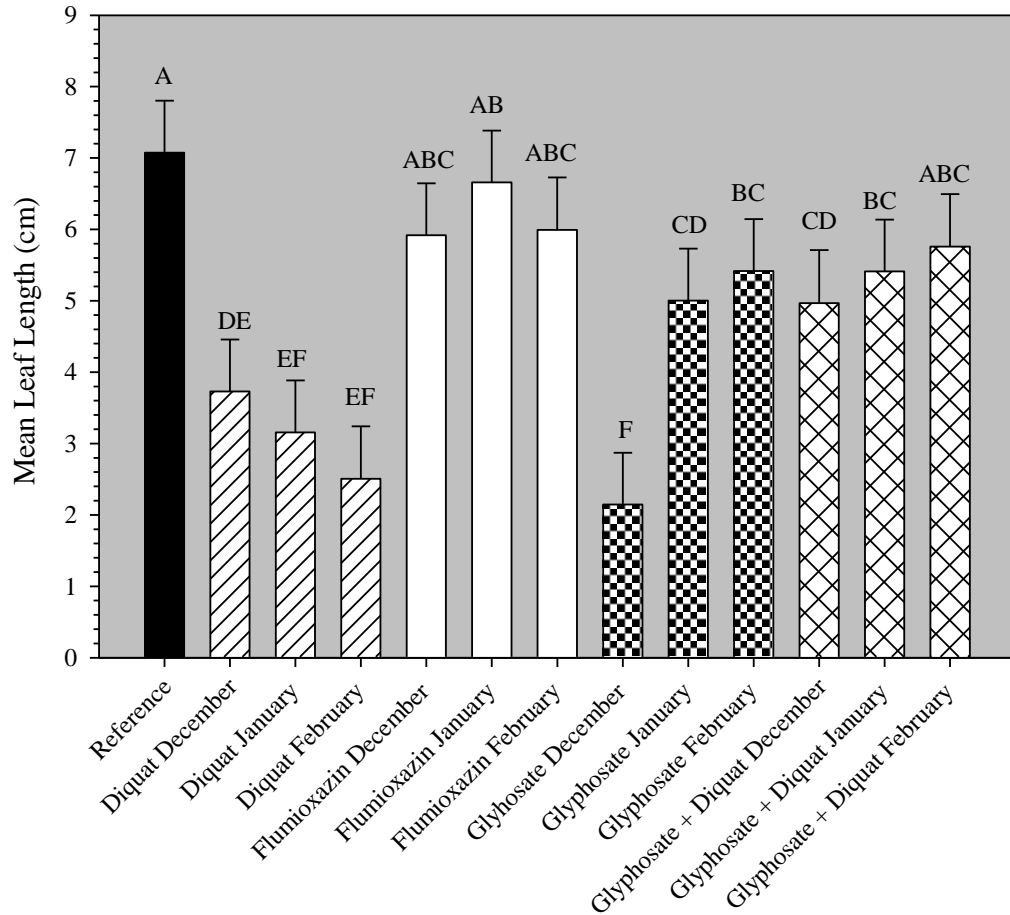


Figure 5 2. Mean leaf length (\pm SE) of bald cypress 20 weeks after bud break in response to winter applied herbicide treatments averaged across treatment and timing. The aquatic herbicides diquat ($1.6 \text{ kg a.i. ha}^{-1}$), flumioxazin ($0.2 \text{ kg a.i. ha}^{-1}$), glyphosate ($3.4 \text{ kg a.e. ha}^{-1}$), and glyphosate ($3.4 \text{ kg a.e. ha}^{-1}$) + diquat ($0.5 \text{ kg a.i. ha}^{-1}$) were applied over the top of bald cypress trees at the equivalent of 94 L ha^{-1} in December, January, or February of 2015-2016 and 2016-2017. Bars with different letters indicate a significant mean leaf length difference at 20 weeks after bud break according to Fisher's Protected LSD test ($p \leq 0.05$); $n = 150$.

tolerance in cotton (*Gossypium hirsutum* L.) has been associated with plant age and restricted herbicide uptake in mature barked stems compared to immature chlorophyllous stems (Ferrell and Vencill 2003, Price et al. 2004). The trees used in the current study would be classified as young (2 to 3 years old) considering the longevity of bald cypress; however, each had fully developed bark. These characteristics may have led to an increased tolerance of flumioxazin applications in the present study.

Leaf length in response to the glyphosate + diquat treatment, was not affected by application timing. Visual injury and shorter average leaf length was documented for glyphosate + diquat treatments compared to the non-treated; however, leaf length was not significant in comparison to flumioxazin treatments 20 WABB. Visual leaf injury symptoms observed in glyphosate + diquat treated trees included abnormal leaf proliferation (i.e. witches' broom), reduced leaf size, chlorosis, and leaf strapping.

Leaf length in response to glyphosate alone was equivalent and greater when applied in January and February compared to December. Glyphosate applications in January and February resulted in average leaf lengths equivalent to the glyphosate + diquat treatments 20 WABB (Figure 5.2). Glyphosate treatments during December severely impacted leaf growth and average length failed to exceed 2.5 cm throughout the study. To date, no literature on the phytotoxicity of glyphosate to dormant bald cypress is available, but it has been documented in other conifer species. Radosevich et al. (1980) reported fall applications of glyphosate did cause minor injury to Douglas fir, white fir [*Abies concolor* (Gord. & Glend.) Lindl], and red fir (*Abies magnifica* A. Murray bis.), but growth was not inhibited. Glyphosate injury in conifers has also been shown to vary considerably due to application timing. Radosevich et al. (1980)

documented that herbicide applications to conifers that were dormant (early spring) or had ceased seasonal growth (fall) were more tolerant to glyphosate applications than conifers exposed during active growing periods (July). King and Radosevich (1985) documented decreased injury when glyphosate was applied to sugar pine (*Pinus lambertiana* Douglas), red fir, white fir, and Douglas fir in September, but injury increased significantly when glyphosate was applied in October. Willoughby (1996) reported no impact on the growth of Douglas fir, Corsican pine (*Pinus nigra* Arnold), lodgepole pine (*Pinus contorta* Douglas), Norway spruce [*Picea abies* (L) Karst.], and Scots pine (*Pinus sylvestris* L.) from high rates of glyphosate applied in March; however, the same herbicide rate applied in January significantly impacted tree growth and survival.

Increased glyphosate injury in December was possibly due to trees not being completely dormant, and some still maintaining leaves, thus allowing the herbicide to contact portions of green leaves, tissue in and around bud scars, or buds that had not completely closed. In cotton, glyphosate is readily absorbed by green stems allowing it to accumulate in fruiting structures leading to floral abnormalities, pollen sterility, and boll abortion (Pline et al. 2002). Leaves of glyphosate treated trees documented severe witches broom characteristics, stunting, and in some instances leaves failed to fully emerge before the conclusion of the trials.

Although diquat is a broad spectrum foliar herbicide, rapid cell death following absorption and acropodial xylem flow, limits translocation from treated leaves (Senseman 2007), thus it was anticipated to provide minimal or no injury to dormant bald cypress trees. Unfortunately, diquat, regardless of timing, severely impacted not only leaf length,

but caused severe non-uniform flushing. Branches exposed to diquat failed to produce more than a couple of leaves per branch and apical shoot meristem dieback was common in the upper auxiliary branches. As a result of the shoot dieback, excess branching from the trunk occurred, possibly to offset reduced leaf production at the auxiliary branches.

Diquat efficacy has been reported in non-deciduous conifer species and is commonly used to control lodgepole pine, mountain pine (*Pinus mugo* Turra), and Douglas fir during winter in New Zealand (Gous et al. 2010). In addition, the closely related compound paraquat has been reported to cause tip dieback and delayed flushing in European beech (*Fagus sylvatica* L.) seedlings (Harmer et al. 2000). Based on the results of the current study and previous research, diquat tolerance in woody plants is species specific and influenced by additional factors such as herbicide rate, timing, and presence/absence of green plant material, and environment conditions at application.

Average leaf length of bald cypress was also influenced by the branch location (top, middle, or bottom) on the tree ($p = 0.0005$) and varied in response to the application (Table 5.1). As expected, the upper branches of the tree canopy came in contact with the herbicide solution more so than the middle and bottom branches, resulting in the top branches exhibiting a mean leaf length (4.24 cm) 14% shorter compared to middle (4.91 cm) and bottom (4.98 cm) branches (Figure 5.3). In addition, a significant treatment by timing interaction was detected in bald cypress non-uniform re-foliation assessment ratings. Bald cypress exposed to flumioxazin in December and January exhibited re-foliation ratings of 1.02 and 1.04, respectively, which was comparable to the 1.00 rating by the reference trees (Figure 5.4). Although February flumioxazin uniformity ratings

(1.46 rating) were significant in comparison to the reference trees (1.00 rating), adverse effects were minimal.

Diquat treatments in December and January resulted in the highest non-uniform re-foliation rating, 4.44 respectively for both treatments, followed by the diquat February treatment (3.88 rating). The high rating in diquat treated trees was the result of branches failing to produce new leaves, high length variability in the leaves that were produced (alternating abnormally long and stunted leaves on the same branch), and excessive trunk branching.

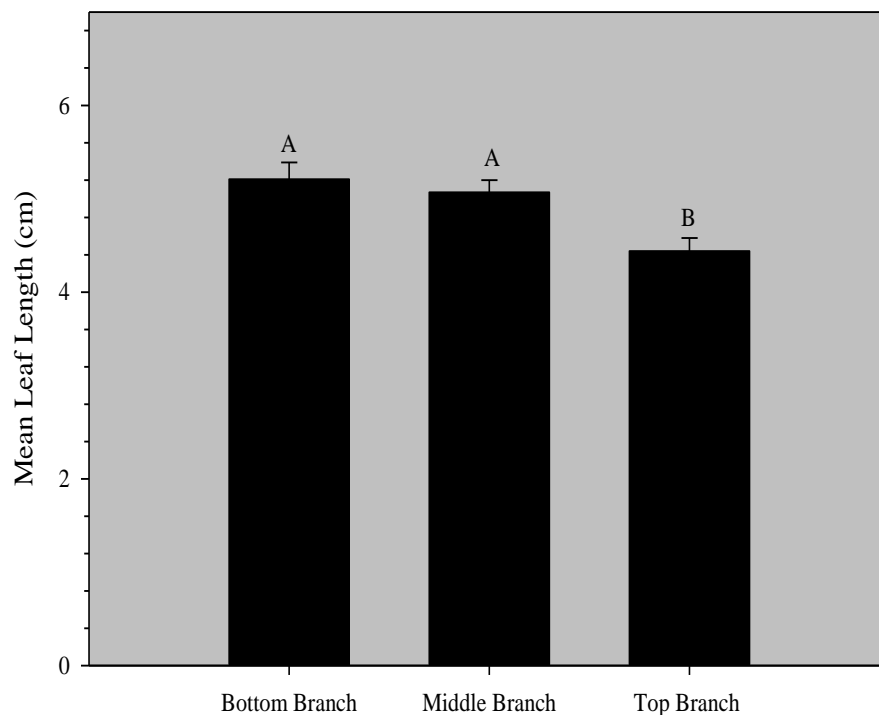


Figure 5 3. Mean (\pm SE) leaf length of bottom, middle, and top branches of bald cypress averaged across all treatments and timings. The aquatic herbicides diquat (1.6 kg a.i. ha^{-1}), flumioxazin (0.2 kg a.i. ha^{-1}), glyphosate (3.4 kg a.e. ha^{-1}), and glyphosate (3.4 kg a.e. ha^{-1}) + diquat (0.5 kg a.i. ha^{-1}) were applied over the top of bald cypress trees at the equivalent of 94 L ha^{-1} in December, January, or February of 2015-2016 and 2016-2017. Bars with different letters indicate a significant mean leaf length difference at 20 weeks after bud break according to Fisher's Protected LSD test ($p \leq 0.05$); $n = 625$.

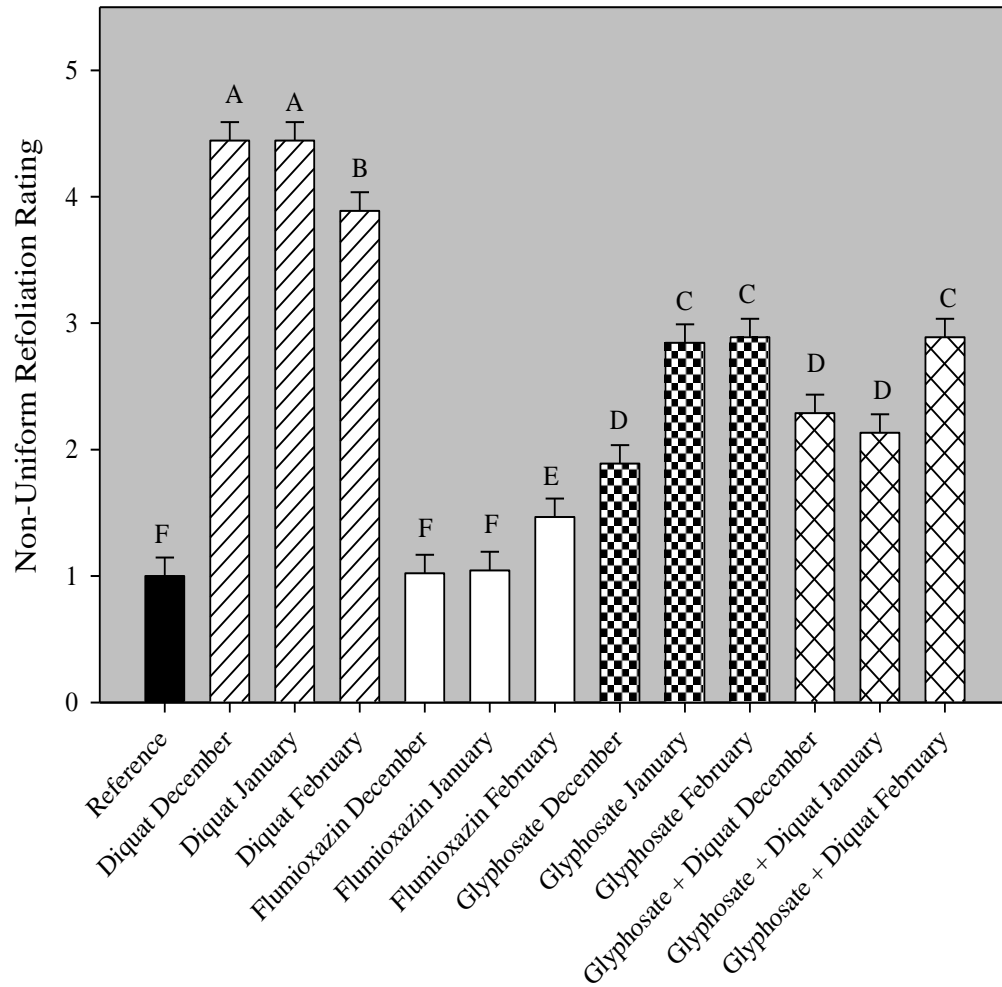


Figure 5.4. Mean (\pm SE) bald cypress re-foliation ratings recorded every 5 weeks over the 20 week observation period following over the top applications of the aquatic herbicides diquat (1.6 kg a.i. ha⁻¹), flumioxazin (0.2 kg a.i. ha⁻¹), glyphosate (3.4 kg a.e. ha⁻¹), and glyphosate (3.4 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) at the equivalent of 94 L ha⁻¹ in December, January, or February of 2015-2016 and 2016-2017. Ratings are based on a scale of 1 to 5: 1 = equivalent to the reference, 2 = slight malformation, 3 = moderate malformation, 4 = moderate to severe malformation, and 5 = severe malformation. Bars sharing the same letter are not significantly different according to Fisher's Protected LSD test ($p \leq 0.05$; $n = 40$).

5.3.2. Giant Salvinia

The ANOVA for giant salvinia biomass revealed a significant treatment ($p = 0.0111$) and treatment by application timing effect ($p < 0.0001$) (Table 5.1). Giant salvinia control was at least 97% when diquat was applied in December and January and control dropped to 56% for the February application (Table 5.2). In contrast, glyphosate + diquat control when applied in December was 72% and increased to at least 92% when applied in January and February. Giant salvinia control was 85 to 98% and equivalent when flumioxazin was applied in December, January, and February and 81 to 90% equivalent for glyphosate applied in January and February.

For December applications, giant salvinia control was 86 to 97% and equivalent for diquat, flumioxazin, and glyphosate, but was 72% for glyphosate + diquat. For January applications, giant salvinia control was at least 96% for diquat, flumioxazin, and glyphosate + diquat, and was 81% for glyphosate alone. Giant salvinia control in response to February applications was 85 to 92% and equivalent for flumioxazin, glyphosate, and glyphosate + diquat, but only 56% for diquat. Glyphosate applied with diquat did not improve giant salvinia control compared to glyphosate alone at any of the application dates. Giant salvinia control with diquat alone was greater than glyphosate + diquat applied in December (97 vs. 72%, respectively), equal to glyphosate + diquat in January (99 and 96% control respectively), but less than that for glyphosate + diquat applied in February (56 vs 92% control respectively). These results are similar to those reported by Mudge et al. (2016) where fall (October) applications of glyphosate + diquat in combination with various adjuvants yielded 83, 72, and 77% control, respectively.

Table 5.2. The response of giant salvinia (LS-mean percent control) to the aquatic herbicides diquat, flumioxazin, glyphosate, and glyphosate + diquat, applied at three timings during the winter 2015-2016 and 2016-2017.

Treatment ^a	Rate (kg a.i. ha ⁻¹) ^b	Giant Salvinia Biomass Reduction (%) ^c		
		December	January	February
Diquat	1.7	97 ab ^d	99 a	56 d
Flumioxazin	0.2	97 ab	98 a	85 abc
Glyphosate	3.4	86 abc	81 bc	90 ab
Glyphosate + Diquat	3.4 + 0.5	72 cd	96 ab	92 ab

^a Treatments included a non-ionic organosilicon surfactant + non-ionic surfactant added at 0.1% and 0.25% v/v, respectively.

^b Glyphosate applied as kg a.e. ha⁻¹

^c Data converted to percent of non-treated reference.

^d Data are pooled across years. LS-Means followed by the same letter are not significantly different according to Fisher's Protected LSD test ($p \leq 0.05$); $n = 10$.

In addition, Mudge and Sartain (2018) documented a 73% reduction of giant salvinia biomass following December applications of glyphosate + diquat. Diquat applied in February provided a 56% biomass reduction compared to the non-treated reference and was the least efficacious of the treatments tested.

Reduced efficacy of diquat applications in February is likely due to the limited amount of healthy plant material present on the upper plant canopy at the time of treatment. These results are in agreement with data from the previous chapter. Giant salvinia exhibited noticeable freeze damage to at least 95% of the exposed fronds by early February, which made it difficult for the herbicide solution to contact healthy, actively growing plant tissue. Glyphosate and glyphosate + diquat applications in February provided 90 and 92% biomass reductions, respectively.

These results indicate that herbicide applications in winter over the top of bald cypress are a practical management technique for controlling giant salvinia. Flumioxazin applied during December and January controlled giant salvinia at least 97% and when applied in February provided $\geq 85\%$ control with little to no negative effects on leaf growth or re-foliation of bald cypress. Although, giant salvinia was controlled at least 97% with diquat in December and January, 81 to 90% with glyphosate in December, January, and February, and 92 and 96% with glyphosate and diquat in January and February, these treatments negatively impacted bald cypress leaf length and re-foliation. None of the treatments tested led to complete tree mortality and could be potential tools for giant salvinia management; however, the probability of bald cypress injury is significantly increased with glyphosate, diquat, and glyphosate + diquat applications.

In order to maximize giant salvinia control, herbicides should be applied during December and January when the majority of the target plant material is actively growing with minimal freeze damage. If infestations are more severe (i.e. multiple plant layers), multiple applications will be necessary. Late winter applications with contact herbicides should be avoided unless a considerable amount of healthy green plant material is present. Giant salvinia control can vary depending upon the environmental conditions present at application and may vary from one year to the next. Future research should examine the products tested in the current research on more mature bald cypress. The trees utilized in the current study were relatively young considering the longevity of a bald cypress, whereas older trees have greater bark development and metabolic capacity and may be more tolerant to the herbicides evaluated in this research. In addition, other

registered aquatic herbicides should be investigated to determine their effects on bald cypress and their utility for giant salvinia control during the winter.

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5.5. Sources of Materials

¹ Miracle Gro® All Purpose Plant Food, The Scotts Company LLC, P.O. Box 606 Marysville, Ohio 43040.

² Tribune™, Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 24719.

³ Clipper™, Valent USA Corporation, P.O. Box 8025 Walnut Creek, CA 94596.

⁴ Roundup Custom™, Monsanto Company, 800 N. Lindbergh Blvd, St. Louis, MO 63167.

⁵ AirCover™, Winfield Solutions, LLC, P.O. Box 64589 St. Paul, MN 55164.

⁶ Aqua-King Plus®, Winfield Solutions LLC, P.O. Box 64589 St. Paul, MN 55164.

⁷ TeeJet®, Spraying Systems Co., P.O. Box 7900 Wheaton, IL 60187.

⁸ Sigma Plot® Version 11.0, Systat Software Inc. San Jose, CA 95131.

Chapter 6. Utilizing Remote Sensing Technology for Monitoring Chemically Managed Giant Salvinia Populations

6.1. Introduction

Giant salvinia (*Salvinia molesta* D.S. Mitchell) is a floating aquatic fern that has spread from its native range in South America to over 20 countries worldwide (Oliver 1993), including the United States (Johnson 1995). The ornamental plant trade is the likely vector for the intercontinental spread of giant salvinia. Botanical gardens and commercial horticulture have been linked to its introduction in Asia, Africa, North America, and Australia (Harley and Mitchell 1981, Nelson 1984, Thomas and Room 1986, Oliver 1993). It thrives in the southern United States, exhibits rapid growth, and forms dense mats that negatively impact water resources immediately after infestation. Extensive giant salvinia growth impedes waterborne navigation, transportation, irrigation, and recreational activities within infested waterbodies (Pimentel et al. 1999). From an ecological and economic standpoint, this free floating fern has the ability to degrade water quality and wildlife habitat, outcompete native plant species, lower property values, and lead to public health concerns (McFarland et al. 2004).

Large scale management techniques such as lake drawdowns, salvinia weevil (*Cyrtobagous salviniae* Calder and Sands) release, and mechanical harvesting are often implemented for giant salvinia management; however, aquatic herbicides are one of the most commonly used and effective management tools (Netherland 2014). Thousands of acres of giant salvinia are chemically managed annually, but management success is difficult to measure quantitatively. Treatment sites can encompass large areas,

upwards of 500 hectares, and certain areas within these sites can be difficult to access or completely inaccessible by boat.

Large scale applications often use spot assessments and visual injury rating scales as a method to quantify herbicide efficacy; however, these can be subjective and biased to the observer (Madsen and Bloomfield 1993). Numerous environmental factors including coastal tide movement, wind, precipitation, water flow, human interactions, and plant decomposition can also impact assumptions of successful or ineffective control. These factors make it difficult for managers to determine if plants remaining within treatment areas are the result of plant recovery or re-infestation from non-treated areas.

Currently, minimal effort has focused on monitoring herbicide efficacy, use patterns, and long term control following operational management of giant salvinia. Widespread plant infestations, diminished funding, and limited trained personnel make it difficult to evaluate post-herbicide application effectiveness. Although small scale studies are beneficial and provide useful information regarding herbicide evaluations (Nelson et al. 2001, Mudge et al. 2016), additional monitoring tools need to be developed to determine if the best herbicides are being utilized in a field setting.

Remote sensing may be a potential monitoring method for assessing herbicide efficacy after field applications. It has become an important tool for wetland resource managers because of limited access and large expanses of aquatic ecosystems (Carter 1982). Remote sensing involves the use of one or more sensors mounted on various platforms [e.g. aircraft, satellite, unmanned aerial vehicles (UAVs)] that collect information based off reflection of electromagnetic (EM) radiation from a particular area or object (Thorp and Tian 2004). The EM spectrum is composed of different forms of

EM radiation that are grouped according to wavelength (Thorp and Tian 2004). For instance, the human eye detects EM radiation in the visible spectrum, which ranges from 400 to 700 nm in wavelength; however, this is only a small percentage of the EM spectrum (Thorp and Tian 2004). Near-infrared light (700 to 1300 nm) makes up another portion of the spectrum and is considered a good indicator of vegetation health. Unfortunately this spectrum is not visible to the human eye (Thorp and Tian 2004).

Optical remote sensors have the ability to detect and quantify reflectance of EM radiation from vegetation, including that in the NIR region (Thorp and Tian 2004). In the visible region of the spectrum, green vegetation often displays very low reflectance and energy transmission due to energy absorption by photosynthetic and plant pigments (Chappelle et al. 1992). In contrast, reflectance and transmittance is typically high in the NIR spectral region (700 to 1300 nm) due to minute absorption of energy by subcellular particles/pigments and scattering at the interfaces of mesophyll cell walls (Gausman 1974, Gausman 1977, Slaton et al. 2001).

The ability of an herbicide to alter the physiological condition of a plant is also assumed to alter the plant's spectral reflectance characteristics (Robles et al. 2010). Temporal herbicide injury and changes of energy reflectance have been documented in terrestrial plants (Adcock et al. 1990). However, little or no research is available that details changes of giant salvinia NIR reflectance in response to herbicide exposure. In aquatics, research has primarily focused on species differentiation (Best et al. 1991, Everitt et al. 2002, 2008, Jakubauskas et al. 2002) and success of biological control methods using reflectance data (Everitt et al. 2005). Everitt et al. (2005) reported that moderate and severe feeding damage in plants infested with salvinia weevils during May

and July had significantly lower near infrared reflectance values in comparison to healthy plants. The mean NIR reflectance of giant salvinia with moderate feeding damage was reported as 16 ± 1.2 and 14.5 ± 0.09 in May and July, respectively (Everitt et al. 2005). Lower values of 9.5 ± 0.4 (May) and 10.3 ± 1.3 (July) were reported for plants with severe feeding damage (Everitt et al. 2005). In contrast, healthy plants in May and July documented a significantly higher mean NIR reflectance of 38.3 ± 2.6 and 35.7 ± 2.6 , respectively (Everitt et al. 2005). In addition, Robles et al. (2010) documented that simulated spectral data from Landsat 5 TM was capable of detecting and predicting herbicide injury on the floating aquatic plant water hyacinth [*Eichhornia crassipes* (Mart.) Solms].

It is important to note that the validation of satellite acquired data requires the collection of ground-truth data to be used as a reference (Kharbouche et al. 2017). These data are often collected by in situ instrumentation (i.e. radiometers, spectrometers) either ground based, hand held, airborne, etc. (Kharbouche et al. 2017) or visual assessments by visiting pre-determined sampling points within an area of interest. High resolution multispectral satellite imagery and ground truth sampling measurements have been used to successfully distinguish giant salvinia from mixed woody and mixed aquatic vegetation in large reservoirs (Everitt et al. 2008). Although this is very beneficial for natural resource managers interested in mapping healthy giant salvinia infestations, management programs could be improved if a reliable low cost monitoring protocol were established that could differentiate changes in plant health following large scale control programs.

The goal of this research was to determine the applicability of using remote sensing to monitor and potentially predict control of giant salvinia treated with a combination of the aquatic registered herbicides glyphosate + diquat in a field scenario. Thus, the objectives of this study were to 1) investigate the relationship between visual percent control ratings of giant salvinia treated with glyphosate + diquat in Saline Lake, LA, and NIR reflectance data from Landsat 7 ETM+ and Landsat 8-OLI satellites 2) investigate the relationship between NIR reflectance data collected from high resolution WorldView-3 satellite imagery and percent biomass reduction in a small scale field application 3) investigate the relationship between NIR reflectance data collected via drone with a low cost NIR sensor payload and percent biomass reduction under controlled experimental conditions, and 4) develop a simple regression model from these data to predict and monitor giant salvinia control when exposed to glyphosate + diquat treatments.

6.2. Materials and Methods

6.2.1. Saline Lake Pilot Study

Visual percent control of giant salvinia was evaluated using point intercept surveys in Saline Lake, LA, during the summer of 2015. Control ratings were recorded at specific points within a 20.2 ha⁻¹ plot of giant salvinia located on the northern shore of Chee Chee Bay in the southwestern portion of Saline Lake (Figure 6.1). Prior to surveying, giant salvinia within the plot was chemically managed on 2 to 4 July 2015. The treatment consisted of a foliar application of glyphosate¹ [3.3 kg acid equivalent (a.e.) ha⁻¹], diquat² [0.5 kg active ingredient (a.i.) ha⁻¹], and 2 surfactants^{3,4} (0.25 and 0.01

% v/v, respectively). Pre-treatment assessment on 1 July 2015, indicated giant salvinia was healthy, mature, and actively growing throughout the plot. Post treatment point intercept surveys were conducted 2, 3, 6, and 10 weeks after treatment (WAT). Surveys were conducted by navigating via boat to equally spaced (50 m) points created in

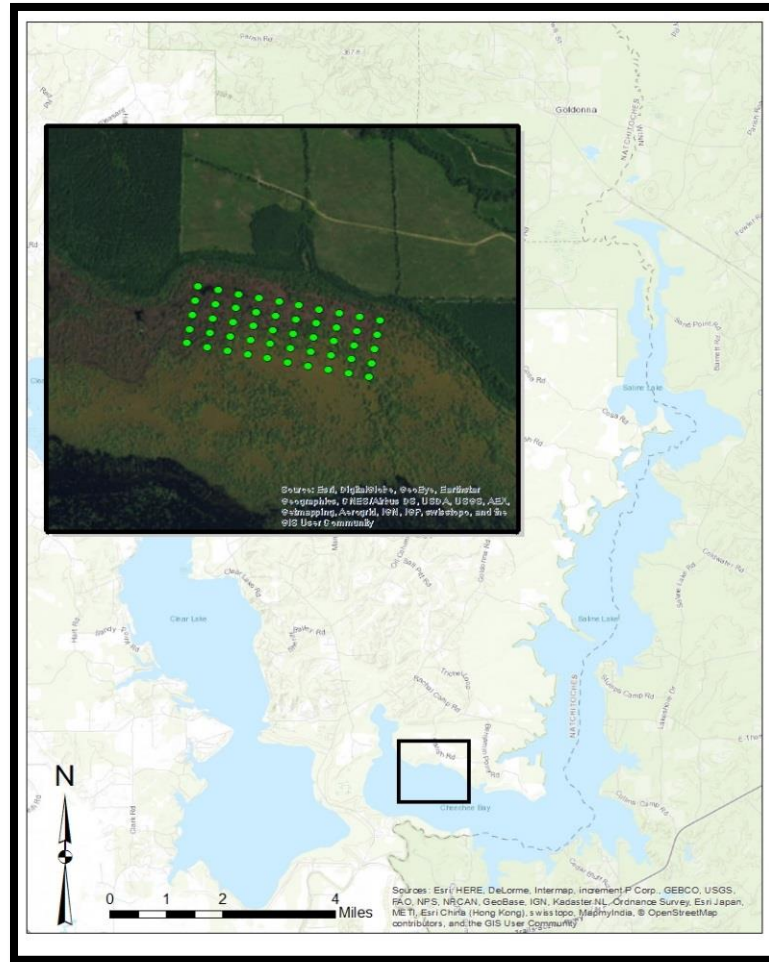


Figure 6.1. The 20.2 ha⁻¹ point-intercept sampling plot on the northern shore of Chee Chee Bay in the southwestern portion of Saline Lake, LA. The points shown in the inserted image represent the locations visited during each survey to evaluate giant salvinia following applications of the aquatic herbicides glyphosate (3.3 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) and 2 surfactants (0.25 and 0.01 % v/v, respectively).

ArcMap⁵ 10.3® Arc GIS computer software, and recording visual percent control at each point. Percent control was determined in 10% increments where 0% = healthy plants/no control and 100% = complete control/no plants present. Data were recorded electronically with a Trimble Yuma™⁶ tablet computer and Farm Works® Farm SiteMate⁷ software version 11.4 (Cox et al. 2011). A total of 50 points were visited during each survey. Landsat-7 ETM+ and Landsat 8-OLI multispectral imagery data were acquired from the U.S. Geological Survey (USGS) EarthExplorer image database. Both Landsat platforms were used to get imagery that was most synchronous to the survey dates. This enabled imagery of the study site to be acquired within 7 days of each point intercept survey (Table 6.1). Previous work by Robles et al. (2010) indicated that the NIR band 4 from Landsat 5 TM consistently related phytotoxicity of herbicides to water hyacinth using a simple regression model; therefore, only NIR bands were used in the analysis. Each image was geometrically corrected and rectified to the World Geodetic Survey 1984 (WGS 84) datum and universal transverse Mercator (UTM zone 15 North) coordinate system. Landsat imagery was corrected to Top of Atmospheric (TOA) reflectance following the instructions listed in the Landsat 7 (USGS 1998) and Landsat 8 Data User Handbook (USGS 2016). Reflectance conversions of all Landsat data were performed using ERDAS (Earth Resource Data Analysis System) Imagine®⁸ software.

NIR reflectance values were extracted using ArcMap Spatial Analyst and averaged to determine the mean NIR reflectance for each 10% increment of visual percent control (Table 6.2). These data were subjected to a simple linear regression model relating percent visual control to NIR reflectance of giant salvinia using

SigmaPlot®⁹ version 11.0 statistical software. A total of 200 points were used in the analysis.

Table 6.1. Point intercept survey dates, number of weeks after treatment each survey was performed, the date of satellite imagery acquisition, and the corresponding sensor platform used to acquire remotely sensed data for Saline Lake, LA, during the summer of 2015.

Survey Date	Weeks After Treatment (WAT)	Satellite Imagery Date	Sensor Platform
20 July 2015	2 WAT	23 July 2015	Landsat Satellite 7 ETM
3 Aug. 2015	3 WAT	31 July 2015	Landsat Satellite 8 OLI TIRS
17 Aug. 2015	6 WAT	16 Aug. 2015	Landsat Satellite8 OLI TIRS
10 Sept. 2015	10 WAT	17 Sept. 2015	Landsat Satellite8 OLI TIRS

Table 6.2. Mean near-infrared reflectance (\pm SE) and the total number of points recorded for each 10% control increment during point intercept surveys following the application of the aquatic herbicides glyphosate ($3.3 \text{ kg a.e. ha}^{-1}$) + diquat ($0.5 \text{ kg a.i. ha}^{-1}$) and 2 surfactants (0.25 and 0.01 % v/v, respectively) in 2015 at Saline Lake, LA.

Percent Control	NIR Reflectance	No. points
	Mean \pm SE	
0	0.2398 ± 0.051	93
10	0.1879 ± 0.011	7
20	0.1872 ± 0.014	10
30	0.1852 ± 0.009	11
40	0.1746 ± 0.009	15
50	0.1610 ± 0.007	23
60	0.1569 ± 0.007	14
70	0.1450 ± 0.013	14
80	0.1111 ± 0.013	9
90	0.0949 ± 0.000	1
100	0.1466 ± 0.011	3
<i>Total = 200</i>		

6.2.2. Small Pond Study

A small pond study was conducted during the summer of 2017 at the University of Louisiana Lafayette Cade Research Farm in Cade, LA. Four adjacent 0.10 ha⁻¹ ponds, 0.3 to 0.6 m in depth, were utilized during the study (Figure 6.2). Each pond was inoculated with giant salvinia during the spring of 2017 and allowed to acclimate until plants achieved 100% coverage of the water surface. WorldView-3 imagery (1 – 1.5 m spatial resolution) of the study site was obtained from the commercial satellite imaging company DigitalGlobe™ through the NextView License agreement with the U.S. Army Engineer Research & Development Center (Vicksburg, MS). DigitalGlobe operates several commercial satellites that are capable of obtaining high spatial resolution images of a particular area with re-visit times every few days.

On 20 June 2017, three of the four ponds were treated with a tank mix of glyphosate (3.3 kg a.e. ha⁻¹), diquat (0.5 kg a.i. ha⁻¹), and surfactant¹⁰ (0.25 % v/v) at a spray volume of 934 L ha⁻¹. Treatments were applied with a handheld spray gun attached to a Kappa¹¹ 55 diaphragm pump. The fourth pond was designated as a reference pond and not treated. Prior to treatment, eight 1.5 x 1.5 m plots, constructed out of PVC pipe, were placed within each pond. Plot area was determined based off the spatial resolution of the WorldView-3 imagery. Plots were anchored to the substrate to prevent movement within ponds. Imagery was acquired on 3 July 2017 (2 WAT) and 28 July 2017 (6 WAT).

It was anticipated that imagery would be acquired a minimum of once every 2 weeks; however, afternoon rain showers and cloudy conditions only allowed the acquisition of images at 2 time periods. Biomass were harvested at 2 WAT and 6 WAT

from multiple PVC plots throughout each pond, transported back to Louisiana State University (LSU), dried (65° C) to a constant weight, and percent control was determined by comparison of biomass in treated plots to biomass in reference plots using the following equation: $(\text{Reference Biomass} - \text{Treatment Biomass}) \div \text{Reference biomass} * 100 = \% \text{ Biomass Reduction}$.



Figure 6.2. World View-3 true color image of the four ponds utilized in the small pond study at Cade, LA. Ponds were treated with the aquatic herbicides, glyphosate (3.3 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) and surfactant (0.25% v/v) on 20 June 2017. Imagery was acquired on 3 July 2017 (2 weeks after treatment). The red arrow indicates a sampling plot in treatment pond 2 surrounded by open water.

WorldView-3 imagery was geometrically corrected to control points using ground measurements and visual targets to ensure the accuracy and location of each plot within the sampled ponds. Multispectral data were processed and corrected to TOA reflectance in ERDAS Imagine software following the user guidelines provided by DigitalGlobe (Kuester 2016). For each sampling period, NIR reflectance and percent control data of each harvested plot were subjected to a simple linear regression using SigmaPlot® version 11.0 statistical software.

6.2.3. Mesocosm Study

On 23 August 2017, giant salvinia was planted at the LSU Aquaculture Research Facility (Baton Rouge, LA) into 30 (76 L) plastic containers (49.5 cm diameter by 58.4 cm height) filled with approximately 60 L of pond water (pH 8.5) and amended with 14 g sphagnum moss to lower the pH to < 7.0 . In addition, 2.1 g of Miracle-Gro®¹² Water Soluble Lawn Food (24-8-16) was applied to each container at planting and every 2 weeks throughout the 8 week study. Plants were allowed approximately 14 days to acclimate to experimental conditions. Plants were healthy, actively growing, and were beginning to form multiple layers at the conclusion of the 2 week acclimation period. Plants in 25 of the 30 tanks were treated with the same herbicide combination utilized in the small pond study with the remaining 5 designated as reference tanks. Biomass were collected from 5 of the 30 tanks at 2 WAT, 4 WAT, 6 WAT, and 8 WAT. Reference tanks and the remaining 5 treated tanks were harvested at the conclusion of the 8 week study. All harvested biomass were dried (65° C) to a constant weight and percent control was determined using the same methods as the small pond study.

Imagery of the study area was collected via a DJI Phantom^{TM13} drone equipped with a low cost Sentra¹⁴ Single SensorTM capable of capturing images in the 575 to 1050 nm wavelength spectrum. AgVault^{TM15} data management software was utilized for autonomous flight control, image acquisition, and mosaicking of acquired images. Images of the study area were acquired prior to biomass collection on cloudless days between 1100 and 1200 hours at a flight altitude of 18.3 m with 80% image overlap. Reflectance targets were placed within the study area and used to calibrate sensor reflectance based off values previously generated using a FieldSpec3®¹⁶ spectroradiometer during appropriate solar and weather windows. Image analysis was performed in ArcMap by over laying a 0.10 m radius polygon feature in the center of each tank, followed by the conversion of each raster image pixel inside the polygon to individual points containing reflectance values using ArcMap Spatial Analyst tools. Polygons were placed in the center of each tank to avoid shadowing or edge effects that may impact pixel values (Figure 6.3).

This process converts individual pixels of the raster image and displays them as individual points. The number of points within each tank polygon ranged between 79 to 81 points. A spatial join was then performed to create a new polygon feature class containing the average reflectance values of each polygon within of each tank. NIR reflectance and percent control data were subjected to a simple linear regression using SigmaPlot® version 11.0 statistical software. The resulting regression equation was then used in conjunction with NIR reflectance values collected during the small pond study at 6 WAT to predict giant salvinia percent control. The predicted percent control values

were then compared to the observed percent control values established in the small pond study at 6 WAT.

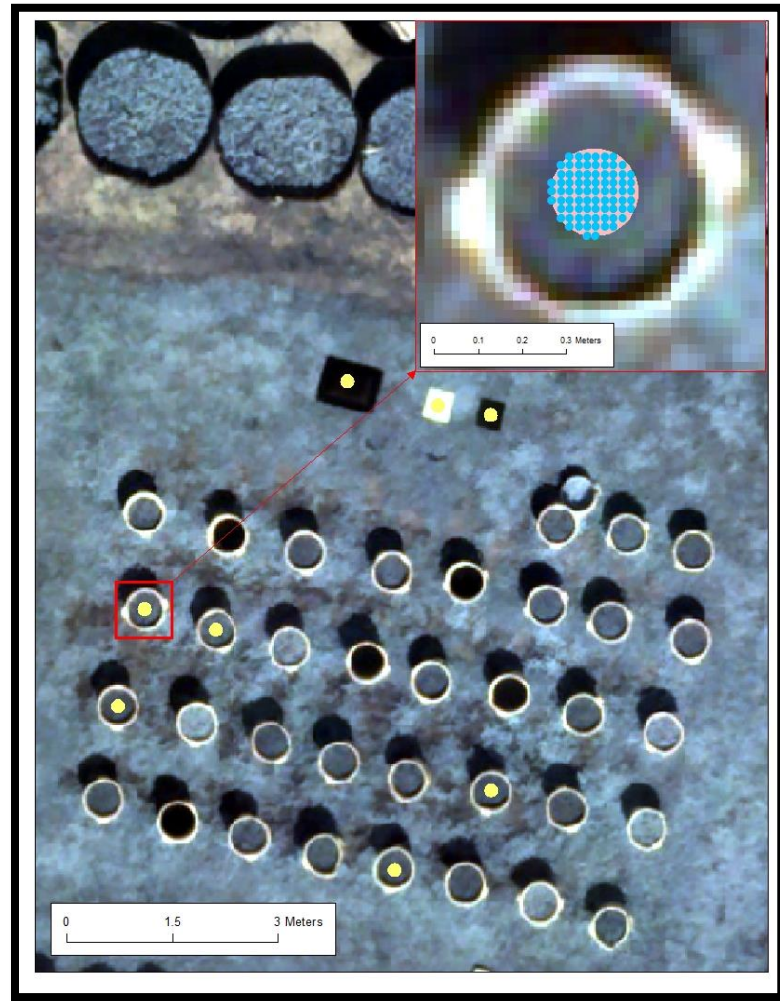


Figure 6.3. Imagery of giant salvinia treated with the aquatic herbicides glyphosate ($3.3 \text{ kg a.e. ha}^{-1}$) + diquat ($0.5 \text{ kg a.i. ha}^{-1}$) and surfactant ($0.25\% \text{ v/v}$) at the Louisiana State University Aquaculture Research Facility in Baton Rouge, LA. Imagery was collected with a DJI Phantom drone and Sentra Single Sensor payload on 5 October 2017 (4 weeks after treatment). Polygons (0.10 m radius) inside the tanks represent the area used for calculating the average near-infrared reflectance of each mesocosm tank. Raster pixels intersecting each tank polygon were converted to points as seen in the inserted image. Near-infrared reflectance data associated with each point were then used to calculate the average near-infrared reflectance within each tank.

6.3. Results and Discussion

6.3.1. Saline Lake Pilot Study

NIR reflectance of chemically managed giant salvinia and visual percent control ratings in Saline Lake, LA, were significant and negatively correlated ($R^2 = -0.785$) (Figure 6.4), thus control decreased as reflectance increased. These data are comparable to Robles et al. (2010) that documented the same response between band 4 (NIR) of Landsat 5 TM and phytotoxicity ratings of water hyacinth treated with glyphosate and imazapyr. Although visual observations and reflectance data documented a negative linear relationship of $R^2 = -0.785$, factors such as sample size, spatial resolution, plant movement, and other co-occurring species likely had an impact on the analysis.

A total of 200 visual observations were used in the analysis, but the number of observations for each percent control increment were not equal. For instance, only three observations documented 100% plant control compared to 22 observations documenting 50% control (Table 6.2). Although satellite imagery was collected within 7 days of each survey, it was not synchronous for the day of surveys. The small number of samples with 100% control ($n = 3$) and the ability of giant salvinia to move into areas that were essentially “open water” between surveying and image collection most likely contributed to the higher average reflectance of the 100% control ratings than that observed for 80 and 90% control. In addition, considerable amounts of healthy bald cypress [*Taxodium distichum* (L.) Rich.] trees were present throughout the sampling plot. Bald cypress,

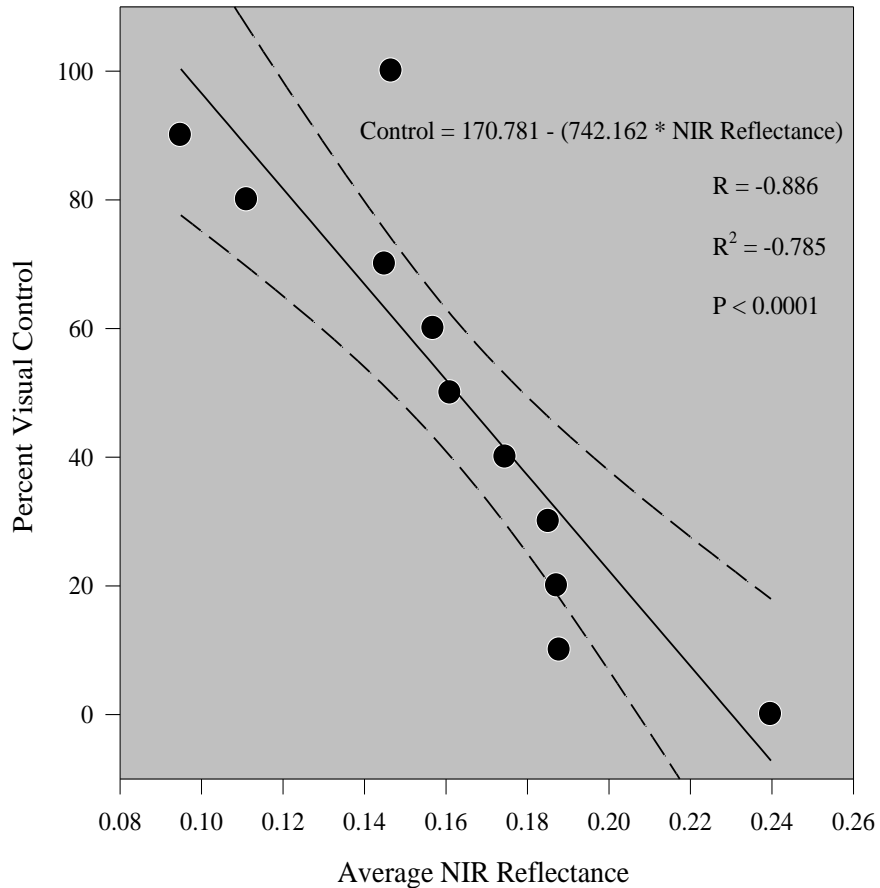


Figure 6.4. Linear relationship of visual control ratings for giant salvinia treated with the aquatic herbicides glyphosate (3.3 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) and surfactant (0.25% v/v) at Saline Lake, LA, and near-infrared reflectance values acquired from Landsat 7 and Landsat 8 satellite platforms. Visual ratings were collected at 2, 3, 6, and 10 weeks after treatment. Point values represent the average NIR reflectance for each 10% visual control rating increment (0 to 100 scale). The solid black line represents the regression line and dashed lines represent regression line 95% confidence intervals.

combined with the low spatial resolution of multispectral Landsat images (i.e. 30 meters) likely had an impact on the true NIR reflectance of giant salvinia within the plot. Despite these limitations, there was a clear distinction between the NIR reflectance of injured and non-injured plants. The availability of Landsat data at no cost makes this technology useful for monitoring management success of giant salvinia in a large scale field scenario.

These results increased the expectations of the applicability of this study; however, it was hypothesized that precision could be improved if tested on a smaller scale.

6.3.2. Small Pond Study

Percent control and NIR reflectance of giant salvinia in sampled plots were significantly correlated at 2 and 6 WAT with $p = 0.047$ and $p < 0.0001$, respectively. Data collected 6 WAT was more representative of actual percent control and documented a stronger negative linear relationship ($R^2 = -0.843$) compared to 2 WAT data ($R^2 = -0.579$) (Figure 6.5). The low rate of diquat ($0.5 \text{ kg a.i. ha}^{-1}$) in combination with glyphosate ($3.3 \text{ kg a.e. ha}^{-1}$) likely influenced the 2 WAT data. Diquat is a fast acting contact herbicide that leads to rapid wilting and desiccation several hours after application and complete foliar necrosis in 1 to 3 days (Senseman 2007), and is commonly used as a marker to indicate previously treated areas. The low rate of diquat likely resulted in a NIR reflectance value not representative of actual percent control at 2 WAT.

Sampling at 6 WAT allowed ample time for treated plants to become necrotic and degrade, thus providing a stronger negative linear relationship between giant salvinia NIR reflectance and percent control within sampling plots. These data are contrasting to those of Roble et al. (2010) that documented the strongest linear relationship between phytotoxicity and reflectance of water hyacinth 2 WAT, when treated with glyphosate and imazapyr. Contrasting results are most likely due to herbicide rate, species being tested, and experimental design. The current study was implemented to simulate a field based application, as opposed to a controlled mesocosm experiment where complete

herbicide to plant contact is easily attainable. In addition, the current study utilized biomass data, as opposed to visual phytotoxicity ratings.

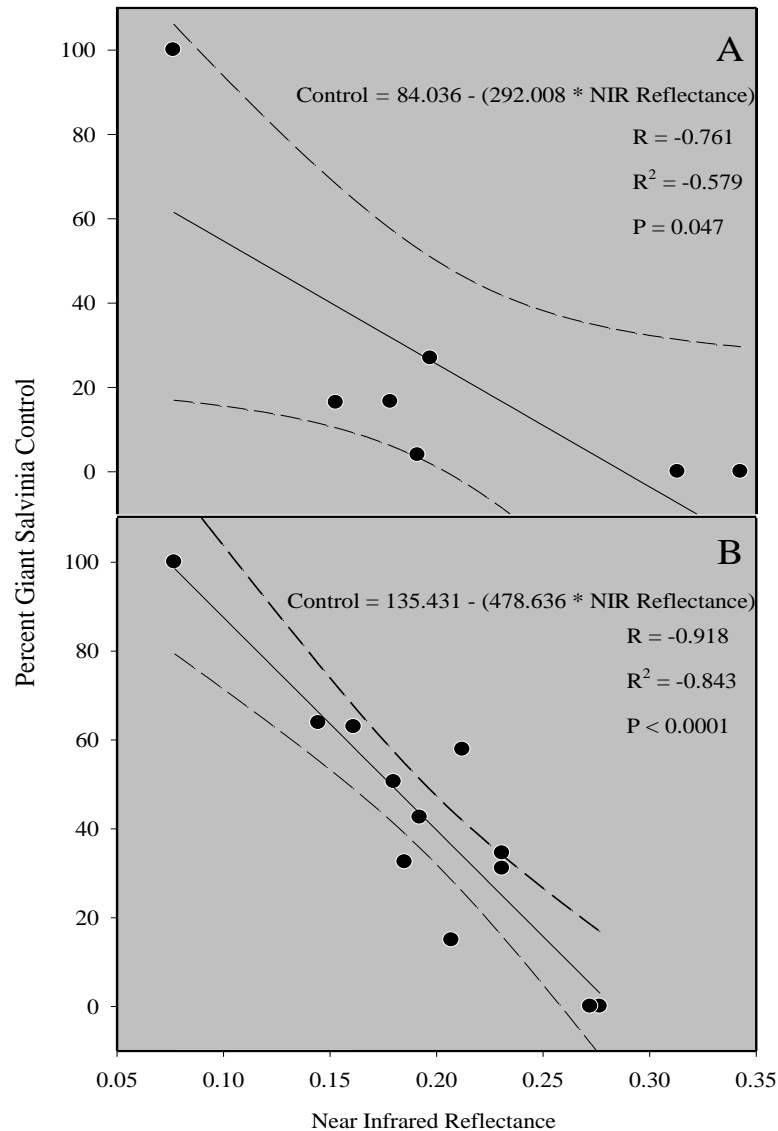


Figure 6.5. Linear relationship between percent control of giant salvinia treated with the aquatic herbicides glyphosate (3.3 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) and surfactant (0.25% v/v) on 20 June 2017 at Cade, LA, and the near-infrared reflectance values acquired from WorldView-3 satellite imagery on A) 3 July 2017 (2 weeks after treatment) and B) 28 July 2017 (6 weeks after treatment). Point values represent percent control of giant salvinia and NIR reflectance values within sampled plots. The solid black line represents the regression line and dashed lines represent 95% confidence intervals of the regression line.

6.3.3. Mesocosm Study

Data collected from the 8 week mesocosm study documented a strong negative linear relationship ($R^2 = -0.914$) between percent control and NIR reflectance values of giant salvinia (Figure 6.6). Because the strongest relationship between percent giant salvinia control and NIR reflectance was documented during this study, the resulting linear regression equation was used to predict percent control values utilizing data collected during the small pond study. The predicted control values were plotted against observed control values to examine the relationship. The following formula, also depicted in Figure 6.6, was used to predict percent control values: Predicted % Control = $104.61 - (306.60 * \text{NIR Reflectance})$. The relationship between predicted and observed percent control values was linear and significant ($p \leq 0.0001$) yielding R and R^2 values of 0.918 and 0.843, respectively (Figure 6.7). Data in all three studies documented a negative linear relationship; thus it is clear that as herbicide control increases the NIR reflectance of the plant canopy decreases. A similar response was documented by Everitt et al. (2005) who documented decreased NIR reflectance values as giant salvinia damage increased in response to the biological control agent salvinia weevil. Everitt et al. (2005) also documented NIR reflectance values of healthy, moderate, and severely damaged giant salvinia to be 35.7 ± 7 , 14.5 ± 0.9 , and 10.3 ± 1.3 , respectively, which are comparable to the results reported in the current mesocosm study that documented 0, 50, and 75% control to correspond to a NIR reflectance of 34.1, 17.9, and 9.7, respectively.

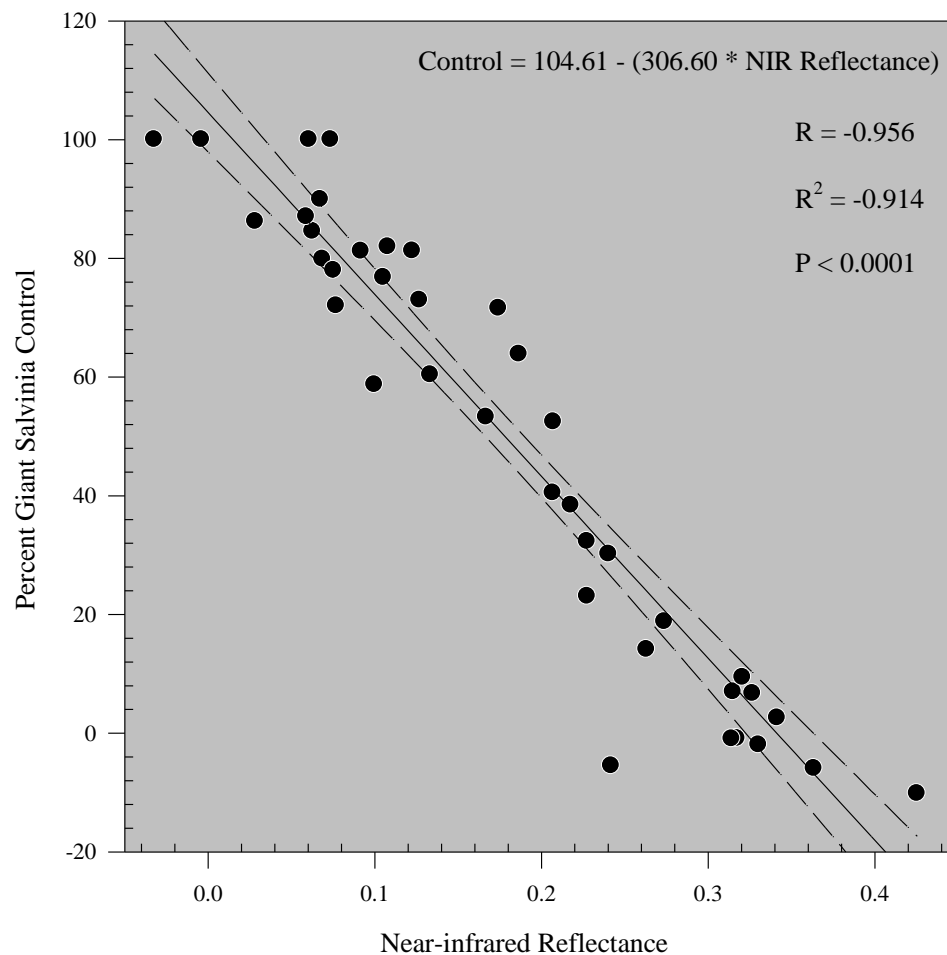


Figure 6.6. Linear relationship between percent control of giant salvinia treated with the aquatic herbicides glyphosate (3.3 kg a.e. ha⁻¹) + diquat (0.5 kg a.i. ha⁻¹) and surfactant (0.25% v/v) on 7 September 2017 and near-infrared reflectance values collected during the mesocosm trial at Baton Rouge, LA. Point values represent percent control established from collected biomass data and NIR reflectance values acquired from a DJI Phantom drone equipped with a Sentera Single Sensor. The solid black line represents the regression line and dashed lines represent 95% confidence intervals of the regression line.

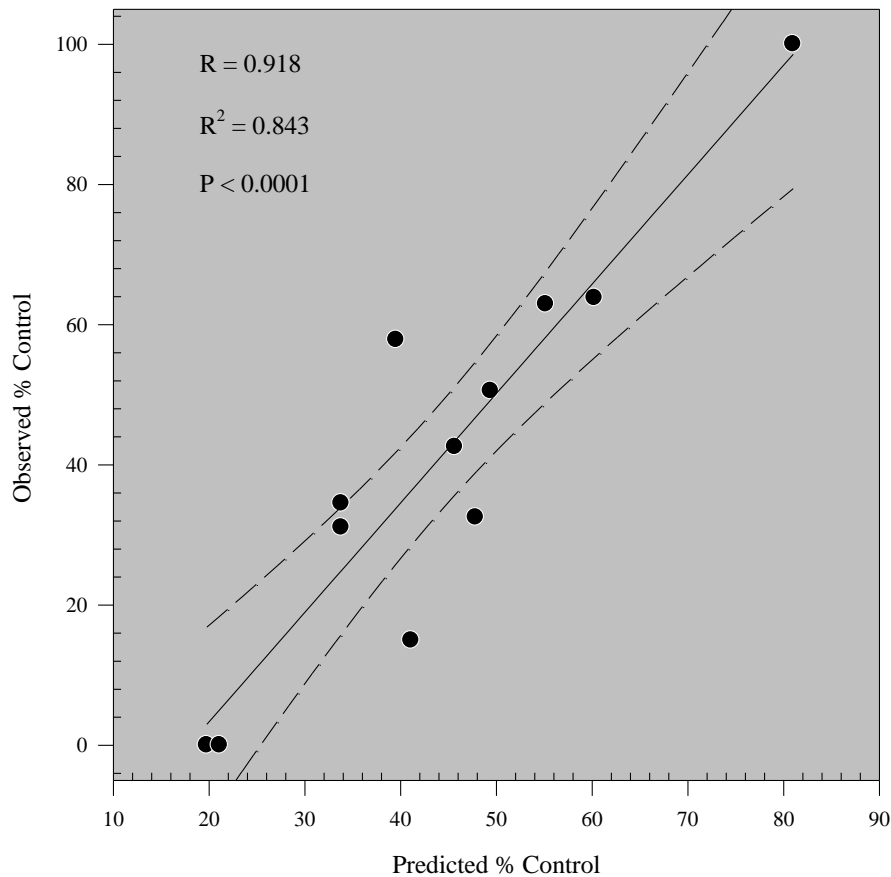


Figure 6.7. Linear relationship between observed % control and predicted % control of giant salvinia treated with the aquatic herbicides glyphosate ($3.3 \text{ kg a.e. ha}^{-1}$) + diquat ($0.5 \text{ kg a.i. ha}^{-1}$) and surfactant ($0.25\% \text{ v/v}$) 6 weeks after treatment at Cade, LA. Predicted % control values represent the estimated percent control using NIR reflectance data from Worldview-3 imagery and the linear regression equation in Figure 6.6. The solid black line represents the regression line and dashed lines represent 95% confidence intervals of the regression line.

The results of these data indicate that it is possible to predict and monitor percent giant salvinia control within treatment areas. Based on the NIR spectral response of giant salvinia to herbicide applications, remote sensing can provide beneficial information on the success of large scale herbicide applications. Estimations of percent control can be determined by using NIR values of a remotely sensed image and the aforementioned equation. Increases or decreases in predicted control values and differentiating between

treated and non-treated plants within an area will provide natural resource managers with critical information about the success of a treatment, potential plant recovery or re-infestation, and the total amount of acreage treated. Future research should evaluate the accuracy of the prediction model on a larger scale and its precision with data collected from other NIR sensors. In addition, research investigating additional spectral bands and/or band combinations may provide more information for monitoring aquatic plant management operations.

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6.5. Sources of Materials

¹ Roundup Custom™, Monsanto Company, 800 N. Lindbergh Blvd, St. Louis, MO 63167.

- ² Tribune™, Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 24719.
- ³ Aqua-King Plus®, Winfield Solutions, LLC, P.O. Box 64589 St. Paul, MN 55164.
- ⁴ AirCover™, Winfield Solutions, LLC, P.O. Box 64589 St. Paul, MN 55164.
- ⁵ ESRI 2017. ArcMap® 10.3 ArcGIS Desktop. Redlands, CA: Environmental Systems Research Institute.
- ⁶ Yuma™, Trimble. Sunnyvale, California.
- ⁷ Farm Works®, Farm Site Mate 11.4, Hamilton, Indiana.
- ⁸ ERDAS, ERDAS® Imagine 2015. Hexagon GeoSpatial, Peachtree Corners Circle Norcross.
- ⁹ Sigma Plot® Version 11.0, Systat Software Inc. San Jose, CA 95131.
- ¹⁰ Turbulence™, Winfield Solutions LLC, P.O. Box 64589 St. Paul, MN 55164.
- ¹¹ Kappa-55 diaphragm pump, UDOR U.S.A, Inc. 500 Apollo Drive, Lino Lakes, MN 55014.
- ¹² Miracle Gro® All Purpose Plant Food, The Scotts Company LLC, P.O. Box 606 Marysville, Ohio 43040.
- ¹³ DJI Phantom 3™. SZ DJI Technology Co. Ltd. Shenzhen, China.
- ¹⁴ Sentra Single Sensor™, Sentra LLC. 6636 Cedar Ave. South Ste 250, Minneapolis, MN 55423.
- ¹⁵ AgVault™ Software, Sentra LLC. 6636 Cedar Ave. South Ste 250, Minneapolis, MN 55423.
- ¹⁶ FieldSpec® 3. Analytical Spectral Devices Inc. (ASD). 2555 5th Street, Suite 100 Boulder, CO 80301.

Chapter 7. Summary and Conclusion

A series of outdoor mesocosm trials were conducted from 2015 until 2017 in Baton Rouge, LA, to investigate alternative giant salvinia (*Salvinia molesta* D.S. Mitchell) management strategies. Herbicide screening trials were conducted to evaluate non-aquatic herbicides to find new products that are efficacious against giant salvinia. The mesocosm trials documented metsulfuron and sulfometuron to be the most effective against the invasive fern. Both herbicides caused plants to become necrotic, lose buoyancy, and desiccate as early as 2 weeks after treatment (WAT) and 100% plant mortality was documented by 8 WAT. In addition, clomazone, halosulfuron, and bensulfuron provided 69, 76, and 77% control, respectively. Herbicide treatments that provided $\geq 30\%$ control in trial 1 (with the exception of clomazone) were re-evaluated in trial 2 at additional rates. All herbicide treatments in trial 2 significantly reduced giant salvinia biomass compared to the non-treated reference. In addition, all three rates of metsulfuron and sulfometuron provided 98 to 99% control. Although metsulfuron and sulfometuron did not provide 100% giant salvinia control in trial 2 at 12 WAT, no new frond growth was observed and harvested material consisted of small rhizome fragments that had little to no viability. The results of these studies conclude that giant salvinia is sensitive to low use rates of metsulfuron [21 g active ingredient (a.i.) ha⁻¹] and sulfometuron (158 g a.i. ha⁻¹) and regrowth of treated plant material is minimal.

Mesocosm trials were conducted on giant salvinia collected from select waterbodies throughout Louisiana to evaluate glyphosate efficacy on giant salvinia collected throughout Louisiana to determine if adequate plant control can still be

achieved with this herbicide. Results from this research suggest that giant salvinia populations in the heavily managed waterbodies of Lake Bisteneau, Caddo Lake, Cross Lake, Saline Lake, Toledo Bend Reservoir, and Turkey Creek Lake are no less susceptible than those where glyphosate has not been applied. There were no differences between populations and no population by glyphosate rate interaction; however, there was a significant glyphosate rate effect. The 4.2 kg acid equivalent (a.e.) ha⁻¹ rate of glyphosate provided better control in comparison to the 3.4 kg a.e. ha⁻¹ rate, resulting in 98 and 95% control, respectively. Only the 4.2 kg a.e. ha⁻¹ rate of glyphosate provided 100% control when applied to the Caddo Lake, Cross Lake, Saline Lake, and non-managed giant salvinia populations. Although plants were arranged as a single layer to encourage complete herbicide to frond contact, giant salvinia was able to persist in several of the populations tested. These results highlight the difficulty of obtaining 100% giant salvinia control even under optimal treatment conditions. Although glyphosate resistance was not conclusive in the present study, there is still cause for concern due to the continuous use of glyphosate, limited management tools, and the high genetic variability within giant salvinia populations.

Outdoor mesocosm trials to investigate the efficacy of the aquatic herbicides glyphosate, diquat, flumioxazin, and glyphosate + diquat against giant salvinia during the winter of 2015-2016 (trial 1) and 2016-2017 (trial 2) documented contrasting results. There was no significant herbicide by timing (December, January, or February) interaction in trial 1 or trial 2. However, significant differences were noted for herbicide treatment and application timing in trial 1. Glyphosate, flumioxazin, and glyphosate + diquat treatments in trial 1 provided 55, 45, and 45% control, respectively. Diquat

applications provided significantly less control and only reduced biomass by 27% of the non-treated reference. In addition, trial 1 herbicide applications during January and February performed significantly better by providing 47 and 50% control, respectively, compared to 33% control achieved during the December application period.

In trial 2, plants were subjected to an extended period of sub-freezing temperatures and as a result giant salvinia control was $\geq 99\%$ and no significant differences were noted for herbicide treatment or application timing. These data provide evidence that successful control of giant salvinia in the winter is possible; however, control will be more dependent on environmental conditions and influenced by herbicide application timing relative to freeze/frost events. Overall, both trials provided some level of control when compared to non-treated plant material.

The herbicides diquat ($1.6 \text{ kg a.i. ha}^{-1}$), glyphosate ($3.4 \text{ kg a.e. ha}^{-1}$), flumioxazin ($0.2 \text{ kg a.i. ha}^{-1}$), and glyphosate ($3.4 \text{ kg a.e. ha}^{-1}$) + diquat ($0.5 \text{ kg a.i. ha}^{-1}$) were evaluated as over the top applications (i.e. simulated aerial application) to determine the impact on dormant bald cypress trees and giant salvinia during December, January, and February of 2015-2016 and 2016-2017. All herbicide treatments reduced giant salvinia biomass 56 to 99%. In addition, flumioxazin applications during December, January, and February provided $\geq 85\%$ giant salvinia control with little or no negative impacts to bald cypress health. A significant treatment by timing interaction was detected for mean leaf length and uniform re-foliation ratings of bald cypress. The treatment by timing interaction revealed that trees exposed to flumioxazin, regardless of application timing, did not result in a significant decrease in average leaf length in comparison to reference trees 20 weeks after bud break (WABB). In addition, bald cypress exposed to flumioxazin in December

and January exhibited a uniform re-foliation rating of 1.02 and 1.04, respectively, which was comparable to the 1.00 rating of the reference trees. Although complete tree mortality was not documented in either study, delayed and abnormal leaf formation, reduced leaf length, irregular canopy formation, or no negative effects were observed among herbicide treated bald cypress. This research suggests that herbicide applications over the top of dormant bald cypress is a practical management technique for controlling giant salvinia.

High resolution multispectral satellite imagery and ground truth sampling measurements have been successful for distinguishing giant salvinia from mixed woody and mixed aquatic vegetation in large reservoirs (Everitt et al. 2008). Although this is beneficial for mapping existing populations and/or detecting new healthy giant salvinia infestations, management programs could be improved if a reliable low cost monitoring protocol were established that could differentiate changes in plant health following large scale herbicide applications. Field sampling data from Saline Lake, LA, documented a negative linear relationship ($R^2 = -0.785$) between Landsat near-infrared (NIR) reflectance data and visual percent control ratings. Additional research utilizing high resolution WorldView-3 satellite reflectance data indicated percent control and NIR reflectance of giant salvinia in sampled plots were significantly correlated at 2 and 6 weeks WAT with $p = 0.047$ and $p < 0.0001$, respectively. However, data collected 6 WAT was more representative of actual percent control and documented a stronger negative linear relationship ($R^2 = -0.843$) compared to 2 WAT data ($R^2 = -0.579$). Additional data collected with a DJI Phantom drone and low cost Sentra Single Sensor during an 8 week mesocosm study documented the strongest negative linear relationship

($R^2 = -0.914$) between percent control and NIR reflectance values. The resulting linear regression equation was then used to predict percent control values utilizing data collected during previous studies. The relationship between predicted and observed percent control values was linear and significant ($p \leq 0.0001$) and yielded R and R^2 values of 0.918 and 0.843, respectively. These data provide strong evidence that based on the NIR spectral response of giant salvinia, following exposure to herbicide applications, remote sensing can provide beneficial information on the success or failure of large scale herbicide applications.

7.1. Literature Cited

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Appendix A. Permission



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STATE OF LOUISIANA
RED RIVER



WATERWAY COMMISSION

Developing the Gift of River Living

March 16, 2016

Consent Form for RRWC Aquatic Research Center

The Red River Waterway District hereby gives its consent for the U. S. Government to take satellite photographs and collect remote sensed data on the following described property located in Natchitoches Parish, Louisiana: Aquatic Research Center (RRWC Owned property) located at Red River Lock and Dam No. 3. The information collected shall be used solely for the purpose of assisting Louisiana State University in studying salvinia molesta. The photography and data collection may only take place between January 2016 and December 2016.

Red River Waterway District

By: Kenneth P. Guidry
Executive Director

Appendix B. Permission




May 30, 2017

Consent Form for Giant Salvinia Remote Sensing Project

University of Louisiana at Lafayette hereby gives consent for the U.S. Government, specifically the U.S. Army Engineer Research and Development Center, to take overhead photographs and collect remote sensed data from ponds 3, 4, 5, and 6 which are situated closest to Parish Road 183 at Cade Farm, located in Cade, Louisiana. The license rights of all overhead photographs and remote sensed data taken in the course of this research project shall adhere to the NextView License terms established in Exhibit "A" (see Exhibit "A", Unclassified Document). All information gathered shall be used solely for the purpose of assisting the U.S. Army Engineer Research and Development Center and Louisiana State University in studying *Salvinia molesta* (see Exhibit "B", "Utilization of Remotely Sensed Data for Assessing Herbicide Efficacy on *Salvinia molesta*").

In connection with this research project, University of Louisiana at Lafayette also hereby gives consent to Louisiana State University AgCenter to enter its premises at Cade Farm solely to maintain the ponds as described in Exhibit "B".

The photography and data collection shall only take place between April 1, 2017 and September 30, 2017.



Dr. E. Joseph Savoie, President
University of Louisiana at Lafayette

5/30/17
Date

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Utilization of Remotely Sensed Data for Assessing Herbicide Efficacy on *Salvinia molesta*

Mr. Bradley Sartain, Graduate Research Assistant, LSU School of Plant, Environmental, and Soil Sciences, Baton Rouge, LA 70803.

Dr. Christopher R. Mudge, Research Biologist, U.S. Army Engineer Research and Development Center, Environmental Laboratory, LSU School of Plant, Environmental, and Soil Sciences, Baton Rouge, LA 70803.

Thousands of acres of giant salvinia (*Salvinia molesta*) are chemically managed annually throughout Louisiana and Texas, but management success is difficult to measure quantitatively. Numerous factors, including but not limited to coastal tide movement, precipitation, water flow, human interactions, and plant decomposition impact assumptions of successful or un-successful control. These factors make it difficult for aquatic plant managers to determine if plants remaining within treatment areas are the result of re-growth or re-infestation. The objective of this research is to evaluate and develop techniques to quickly and accurately assess giant salvinia treatment efficacy in a field scenario utilizing remotely sensed data acquired from high spatial resolution satellite imagery.

The proposed project aims to utilize 4 ponds at the University of Louisiana at Lafayette Cade Research Farm from March 15 to September 31, 2017. Ponds 3, 4, 5, and 6, which are located closest to Parish Road 183, will be infested with giant salvinia and treated with aquatic herbicides. After herbicide application, imagery/data will be collected from the commercial satellite imaging company DigitalGlobe to assess efficacy. Dr. Mudge's federal employer, US Army Engineer Research & Development Center (USERDC), has a NextView License agreement with DigitalGlobe and Mr. Sartain (LSU Graduate Student) will analyze the data. DigitalGlobe operates several commercial satellites including WorldView-2 and WorldView-3 that are capable of obtaining high spatial resolution (1 – 1.5 m) images of a particular area with re-visit times every few days. The high resolution and quick re-visit times of the WorldView-2 and WorldView-3 satellites may allow for a quantitative sampling method to be developed based off the reflectance values received from giant salvinia at different light spectrums. It has been demonstrated that these data can be used to identify different plant species and are capable of monitoring plant damage/stress due to external factors.

The LSU AgCenter and USAERDC are requesting consent to obtain imagery of these 4 ponds strictly for research purposes, with no expectations or requirements from ULL. The ponds will be maintained by the LSU AgCenter throughout the course of the project. After project completion, the ponds will be returned to their current status. All the details regarding the data acquisition and licensing are included in the NextView License agreement form.

Vita

Bradley Sartain, a native of Madison, Mississippi, enrolled in Mississippi State University in 2006 and earned a Bachelor of Science degree in wildlife, fisheries, and aquaculture with a concentration in fisheries science in May of 2011. Shortly after, he entered the graduate program at Mississippi State University in June of 2011 and received a Master of Science degree in agriculture in May of 2014. Over the next year, Bradley worked as a biologist for a lake and pond management company in North Alabama. Working in private industry was a rewarding experience that gave him the motivation to return to graduate school to obtain his doctorate in agronomy specializing in aquatic weed management. After graduation with a Ph.D. from Louisiana State University in 2018, he will pursue a job in the field of aquatic plant management.